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# THE ANTICHOLINESTERASE ACTIVITY IN VITRO OF THE INSECTICIDE PARATHION (p-NITROPHENYL DIETHYL THIONOPHOSPHATE)[1]

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Received for publication April 12, 1950

In recent years German chemists have synthesized a number of esters of phosphoric acid which have proved to be extremely potent inhibitors of cholinesterase (ChE) enzymes. These organic phosphates include di-isopropyl fluorophosphate (DFP), hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), and p-nitrophenyl diethyl thionophosphate (parathion). DFP and TEPP have found clinical application in a number of situations in which cholinergic drugs are employed, including the treatment of abdominal distention, glaucoma, and myasthenia gravis. HETP, TEPP,[2] and parathion,[3] especially the latter, have been employed as insecticides, chiefly in agriculture, and have been more effective than DDT against most insect parasites, including mites. The properties and effects in man of DFP and TEPP have been described in previous communications (1–5). The purpose of the present communication is to outline some of the properties of parathion.

Parathion is a colorless liquid with a characteristic acrid, mercaptan-like odor suggestive of garlic. Its specific gravity is 1.26. Its solubility in water is low, being approximately 0.002 Gm./100 cc. at 25°C. (6). Its volatility at 25°C. and 760 mm. of mercury is also low, being 0.0025

[1] The work described in this paper was carried out under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

[2] Trade names for TEPP insecticide: "Nifos-T" and "TEPP".

[3] Trade names for parathion insecticide, which is now widely used in agriculture, include: "Lethalaire G-54 Parathion Aerosol", "Chipman Parathion", "P. A. R. Parathion", "Phos Kit Parathion", "Paradust Parathion", "Dow Parathion", "Vapophos Parathion", "Penphos Parathion", "Aphamite Parathion", "Parathion Insecticides", "Genithion Parathion", "Edco 15 Parathion", "Niran (Parathion)"; "Geigy Parathion", and "Thiophos (Parathion)".

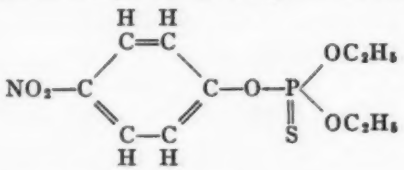
mg./L. of air. Of particular interest is the very slow rate of hydrolysis of parathion in water at any pH below 10, 120 days being required for 50 per cent hydrolysis at 25°C. This is reduced to 8 hours in saturated sodium hydroxide (7). The solubility and rate of hydrolysis of

TABLE 1

*Some properties of organic phosphate antiChE compounds*

| | Molecular weight | Specific gravity | Volatility at 25°C., 760 mm. (mg./L. of air) | Odor | Solubility in water at 25°C. (g./100 cc.) | Half hydrolysis time at 25°C., pH 7 | Acute toxicity to rats $LD_{50}$ (mg./kg.) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Intraperitoneal | Oral | Reference |
| TEPP | 290 | 1.19 | 0.0465 | None | Freely miscible | 8 hrs. | 0.6 | 1.4 | (8) |
| DFP | 184 | 1.055 | 8.16 | Slightly pungent | 1.5 | 16 hrs. | | 6 | (9) |
| Parathion | 291 | 1.26 | 0.0028 | Like garlic | 0.002 | 120 days | Males 7 \| Females 4 | Males 15 \| Females 6 | (1) |

parathion in water are much lower than in the case of DFP or TEPP (Table 1). The formula of parathion is represented below:




Studies performed following the administration of parathion to experimental animals have shown that most of the pharmacological effects of this compound can be explained in terms of its anticholinesterase (antiChE) action (10, 11).

## INHIBITION OF ChE ENZYMES BY PARATHION

The inhibition of the ChE enzymes of human plasma, red blood cells, muscle, and brain by parathion in vitro is recorded in Figure 1

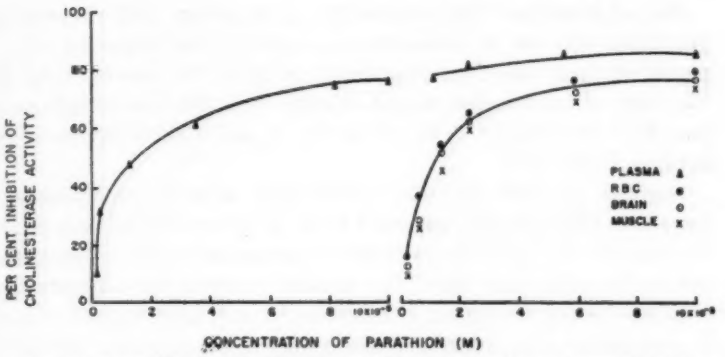



FIG. 1. The inhibition of plasma, red blood cell, brain, and muscle ChE activity by parathion in vitro. The concentrations of the enzyme preparations and methods employed are recorded in Table 2.

TABLE 2

*The sensitivity of human plasma, red blood cell, brain, and muscle cholinesterases to inhibition by parathion in vitro, compared to inhibition by TEPP, DFP, and neostigmine*

The molar concentration of each antiChE agent is recorded which produced 50 per cent inhibition of the ChE activity of a solution or homogenate which hydrolyzed 4.78 $\times 10^{-4}$ mM acetylcholine bromide per hour in the absence of inhibitor. This rate of hydrolysis was produced by 51 mg./ml. of plasma, 124 mg./ml. of brain (cerebral cortex) homogenate, 58 mg./ml. of muscle (psoas) homogenate, and 34 mg./ml. of red blood cell contents. The initial concentration of acetylcholine bromide was 0.015 M. The ChE and antiChE compounds were mixed one hour before the addition of acetylcholine and sodium bicarbonate and the manometric determination of ChE activity (1). ChE activity was also determined by measurement of the increase in acidity that occurred in the presence of phosphate buffer (12), with similar results.

| | PLASMA ChE | BRAIN ChE | MUSCLE ChE | RED BLOOD CELL ChE |
|---|---|---|---|---|
| TEPP..... | $5 \times 10^{-9}$ | $3.2 \times 10^{-8}$ | $3.5 \times 10^{-8}$ | $3.5 \times 10^{-8}$ |
| DFP..... | $9.5 \times 10^{-9}$ | $3 \times 10^{-7}$ | $2.5 \times 10^{-7}$ | $4 \times 10^{-7}$ |
| Neostigmine..... | $4 \times 10^{-8}$ | $4 \times 10^{-7}$ | $3 \times 10^{-7}$ | $8 \times 10^{-7}$ |
| Parathion..... | $1.5 \times 10^{-8}$ | $1.3 \times 10^{-6}$ | $1.5 \times 10^{-6}$ | $1.2 \times 10^{-6}$ |

and Table 2. Plasma ChE was more sensitive to inhibition by parathion than were the ChE enzymes of the red blood cells, muscle, and brain, which were less, and approximately equally sensitive to inhibition.

*Rate of inhibition:* The inactivation of the above ChE enzymes by parathion was not an instantaneous reaction, but occurred over a period of time, reaching a maximum in 20 to 60 minutes (Fig. 2). The rate of inactivation varied directly with the concentrations of parathion and ChE (A > B > C in Fig. 2), and with the temperature, between 5° and 37°C.

*Duration of inhibition:* The combination between parathion and the above ChE enzymes appeared to be partly reversible for a period of several hours after the addition of parathion to the enzyme, and irreversible after that time. The relation between the concentration of parathion and the degree of inhibition of each ChE enzyme (Fig. 1) is compatible with inhibition which was at least partly reversible during the period of two hours which preceded the determination of enzyme activity. The solubility of parathion was not a limiting factor in this experiment.

The partial reversibility of the inhibition of ChE by parathion during approximately the first three hours of their reaction is also demonstrated by the partial restoration of ChE activity (B > A in Fig. 2) that occurred following dilution of the parathion-ChE mixture (of B) during this time. If the inactivation of ChE (in A and B) by parathion had been entirely irreversible during the period of incubation prior to dilution, dilution (of B) would not have affected the per cent inhibition of ChE activity. Since dilution (of B) did result in an increase in active ChE, it must have caused dissociation of some of the parathion-ChE combination. The effect of dilution on ChE activity diminished progressively over a period of three hours, indicating that more and more ChE was being irreversibly inactivated during this time. The progressive irreversible inactivation of ChE during the first hour of this reaction is also reflected by the more rapid rate of inactivation which occurred when the initial concentration of parathion and ChE were higher (B > C). If some irreversible inhibition had not occurred during this time there would have been no difference (between B and C) in the per cent inhibition of ChE activity after dilution (of B), since at that time the concentrations of ChE and of parathion were the same. After about one hour of incubation the inhibition of ChE was approximately the same (in B and C), indicating that by that time irreversible inactivation of ChE was approaching a maximum in both. The rate of irreversible inac-

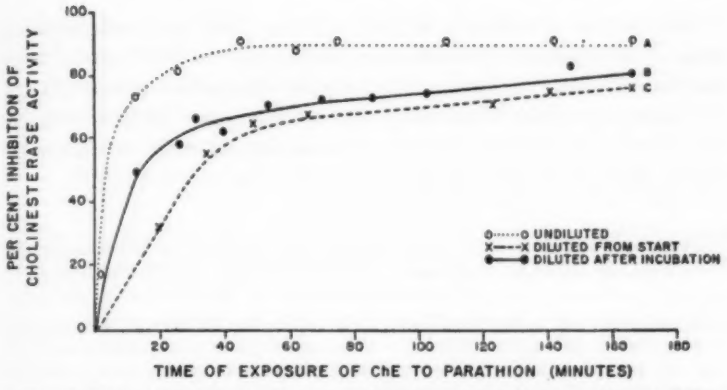



FIG. 2. The rate of inactivation of red blood cell ChE by parathion at 37°C., and the effect of the concentration of parathion and of dilution of the parathion-ChE mixture.

Acetylcholine and sodium bicarbonate were added to aliquots for the manometric determination of ChE activity (1) after incubation of parathion with red blood cell solution for varying periods of time at 37°C. The final concentrations of acetylcholine and of ChE after dilution (B and C) were the same as in Table 2, while in the aliquots which remained undiluted (A) the final concentrations were fourteen times as great.

| PARATHION AND ChE | UNDILUTED | DILUTED 14 TIMES | |
|---|---|---|---|
| | Symbol | | |
| | (A) O·····O | After incubation (B) ●——● | From the start (C) X----X |
| Parathion conc. (M) during incubation | $3.3 \times 10^{-4}$ | $3.1 \times 10^{-4}$ | $2.3 \times 10^{-5}$ |
| Parathion conc. (M) when ChE was determined | $2.5 \times 10^{-4}$ | $2.1 \times 10^{-5}$ | $2.1 \times 10^{-6}$ |

The initial concentrations of parathion and of ChE in A and B were the same, and were fourteen times lower in C. After incubation for periods of time up to three hours aliquots of B were diluted fourteen fold and acetylcholine added for determination of ChE activity, while acetylcholine was added to aliquots of A and C with almost no further dilution, so that the final concentrations of parathion and of ChE were the same in B and C, and were fourteen times higher in A.

tivation of ChE by parathion varied directly with the concentration of parathion and with the temperature, between 5° and 37°C.

The partial restoration of ChE activity that occurred following dialysis of parathion-ChE mixtures during the first few hours of their reaction (Table 3) is further evidence for the partial reversibility of the parathion-ChE combination during this time. This restoration was always incomplete, and the reversibility decreased over several hours, being no longer demonstrable after nine hours at 25°C.

TABLE 3

*The effect of prolonged standing and of dialysis on the inhibition of cholinesterase (red blood cell and brain) by parathion, and comparison with TEPP, DFP, and neostigmine*

Acetylcholine and sodium bicarbonate were added for the determination of ChE activity after the indicated periods of time. The concentrations of acetylcholine and ChE were the same as in Table 2. The presence of toluene (0.25 cc.) completely inhibited bacterial growth. Dialysis against running tap water was begun one hour after the addition of the antiChE compounds to ChE. The effect of dialysis demonstrated the partial reversibility of the combination between parathion and ChE, and between TEPP and ChE, the irreversibility of the DFP-ChE combination, the complete reversibility of the neostigmine-ChE combination, and the partial protection of ChE against parathion by neostigmine.

| | CONCENTRATION (M) | ChE | PER CENT INHIBITION OF ChE ACTIVITY AFTER STANDING AT 23°C. FOR | | |
|---|---|---|---|---|---|
| | | | 1 hr. | 24 hrs. | 24 hrs. (with dialysis) |
| Parathion | $2 \times 10^{-4}$ | Brain | 82 | 83 | 61 |
| Parathion | $2 \times 10^{-4}$ | R.B.C. | 88 | 91 | 68 |
| TEPP | $9 \times 10^{-7}$ | " | 94 | 78 | 60 |
| DFP | $7 \times 10^{-6}$ | " | 92 | 97 | 90 |
| Neostigmine | $3 \times 10^{-4}$ | " | 93 | 93 | 0 |
| Neostigmine and Parathion | $3 \times 10^{-4}$ $2 \times 10^{-4}$ | " | 96 | 98 | 37 |

In the absence of dilution or dialysis, i.e. when parathion-ChE mixtures were merely allowed to stand in aqueous solution, there was progressive inactivation of the enzyme, and restoration of enzyme activity did not occur even after prolonged standing (Table 3). When blood was withdrawn from subjects in whom exposure to parathion had produced a depression of plasma and red blood cell ChE activity 24 or more hours previously, neither dialysis, dilution, nor prolonged standing of the plasma or hemolyzed red blood cells resulted in any restoration of activity.

*Protection of ChE by neostigmine:* When ChE was in combination with neostigmine it was at least partly protected against inactivation by the addition of parathion, as indicated by the restoration of the ChE to near its initial activity when the neostigmine and parathion were removed by dialysis (Table 3).

## SOLUBILITY OF PARATHION IN AQUEOUS AND LIPOID MEDIA

The solubility of parathion in an aqueous and in a lipoid medium was compared by homogenizing an aqueous solution of parathion with an equal volume of peanut oil, removing the peanut oil by centrifugation, and determining the antiChE activity of the remaining

TABLE 4

*The water-lipoid partition of parathion compared with that of TEPP, DFP, and neostigmine*

The concentrations of ChE enzymes and of antiChE compounds were the same as in Table 2. The per cent of each antiChE compound extracted from aqueous solution by the peanut oil is recorded.

| | PLASMA ChE | BRAIN ChE | MUSCLE ChE | RED BLOOD CELL ChE |
|---|---|---|---|---|
| TEPP | 0 | 9 | 14 | 0 |
| DFP | 84 | 85 | 87 | 90 |
| Neostigmine | 11 | 20 | 15 | 16 |
| Parathion | 98 | 94 | 94 | 92 |

aqueous solution. Ninety-two to ninety-eight per cent of the parathion in the aqueous phase was removed by the peanut oil (Table 4), and could be demonstrated quantitatively in the latter by the effect of the peanut oil on the ChE activity of plasma and red blood cells when injected into rats. The addition of a detergent (polyoxyethylene sorbitan monooleate (Tween −80), 2.5 to 5 $\times$ $10^{-3}$M) to *solutions* of parathion did not result in greater inhibition of any of the ChE enzymes.

## DISCUSSION

The degree of inhibition of the ChE enzymes by antiChE compounds in vivo depends on several factors:

1. *The sensitivity of the ChE enzymes to inhibition by the antiChE agent, and the rate at which this inhibition occurs:* Parathion is an active

inhibitor of human ChE enzymes, but is only about one thirtieth as potent as DFP and neostigmine in vitro, and one three hundredth as potent as TEPP (Table 2). All four antiChE compounds inhibit plasma ChE more readily than the ChE enzymes of red blood cells, brain, and muscle, which are less and approximately equally sensitive to inhibition by each compound in vitro. The rate of inhibition of ChE by parathion is considerably slower than the rate of inactivation by neostigmine (13), and moderately slower than the rate of inactivation by DFP (14) or TEPP (5). On the basis of these findings alone the toxicity of parathion might be expected to be lower than that of the other antiChE compounds, and the latent period between absorption and the onset of symptoms might be expected to be longer. The toxicity of parathion in experimental animals after oral or parenteral administration is actually nearly as great as that of DFP, and one tenth that of TEPP (Table 1). Since the differences in the antiChE activities of these compounds in vitro are of a higher order, it is probable that additional properties may influence their toxicity.

2. *The accessibility of the antiChE compound to the ChE enzyme of each important tissue (especially the central nervous system)*: The solubility of neostigmine derivatives (15), DFP, and TEPP in lipoid media appears to be an important factor in determining their accessibility to the ChE enzyme in the central nervous system, and their central effects, presumably because of the high lipoid content of the central nervous system (5). Neostigmine, which is very much more soluble in aqueous than in lipoid media, has very little effect on the central nervous system in vivo (3). TEPP is also more soluble in aqueous than in lipoid media, but has definite central effects when in sufficient concentration, perhaps because of its very marked antiChE activity (5). DFP, which is more soluble in lipoid than in aqueous media, has relatively greater central effects than does TEPP (3). Parathion is less soluble in aqueous medium than the other antiChE compounds (Table 1) and has a greater partition in lipoid medium (Table 4). These solubility properties of parathion would lead one to expect a potent effect of this compound on the central nervous system in vivo.

3. *The duration of inhibition of the ChE enzymes by the antiChE compound:* This depends upon the rate of removal of the antiChE

agent in the body, the reversibility of the ChE-antiChE combination, and the rate of restoration of the ChE enzymes in the various tissues. The removal of the antiChE agent in the body may be by hydrolysis, adsorption on proteins other than the ChE enzymes of important tissues, other means of detoxification, and excretion. The rate of hydrolysis of parathion in water is very much slower than that of DFP or TEPP. In the presence of tissue extracts hydrolysis of all three of these esters of phosphoric acid is accelerated by hydrolytic enzymes, but parathion is the most slowly hydrolyzed. When ChE enzymes that had been inactivated by parathion were allowed to stand in aqueous solution for 24 or more hours there was no restoration of ChE activity (Table 3). In contrast, when ChE that had been inactivated by TEPP was allowed to stand for this period there was some restoration of activity. This difference is apparently due to the very much slower rate of hydrolysis of parathion than of TEPP. The hydrolysis of TEPP (but not of parathion) during this time resulted in shifting of the equilibrium in the direction of the dissociation of the ChE-antiChE combination and the liberation of free ChE. The slow rate of hydrolysis of parathion, even in the presence of tissue fluids, would be expected to play a part in the prolongation of its physiological effects. The extremely slow rate of hydrolysis in water would also lead one to expect the persistence of parathion on agricultural products for long periods of time, in spite of weathering.

The combination between parathion and ChE appears to be partly reversible for a period of several hours after exposure of the enzyme to the inhibitor. This resembles the partial temporary reversibility of the TEPP-ChE combination (5), and is intermediate between the extremely transient reversibility of the DFP-ChE combination (14) and the complete lasting reversibility of the neostigmine-ChE combination (13) (Table 3). This important property of parathion would lead one to expect that the duration of its physiological effects and the rate of restoration of ChE enzymes in the tissues after their depression by this compound might be similar to that found in the case of TEPP (5).

## SUMMARY

1. Parathion (p-nitrophenyl diethyl thionophosphate) inhibits human cholinesterase (ChE) enzymes in vitro, but its activity is less

than that of tetraethyl pyrophosphate (TEPP), di-isopropyl fluorophosphate (DFP), or neostigmine. All of these antiChE compounds have a greater affinity for plasma ChE than for red blood cell, brain, and muscle cholinesterases.

2. The rate of inactivation of ChE by parathion is slower than the rate of inactivation by the other antiChE compounds.

3. The combination between parathion and ChE is partly reversible for several hours and then irreversible. In this regard parathion resembles TEPP, and is intermediate between DFP, which rapidly combines with ChE irreversibly, and neostigmine, which combines with ChE in a completely reversible manner. When ChE is in combination with neostigmine it is at least partly protected against inactivation by parathion.

4. Parathion is much less soluble in water than the other antiChE compounds, and has a greater partition in lipoid as compared to aqueous medium. It is much more slowly hydrolyzed in water than TEPP or DFP.

It is a pleasure to acknowledge the valuable technical assistance in this study of Miss Barbara Ziegler and Mr. Stanford P. Hampton.

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# THE TOXIC EFFECTS IN MAN OF THE ANTICHOLINESTERASE INSECTICIDE PARATHION (p-NITROPHENYL DIETHYL THIONOPHOSPHATE)[1]

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Parathion[2] (p-nitrophenyl diethyl thionophosphate) has been widely used as an insecticide during the past year, and important problems have arisen because of its toxicity for man. Studies performed in experimental animals have shown that most of the pharmacological effects of this compound can be explained in terms of its anticholinesterase (antiChE) action (1, 2). Studies performed in vitro have shown that it is poorly soluble in water, highly soluble in lipoid media, very slowly hydrolyzed in water, that it combines relatively slowly with the cholinesterase (ChE) enzymes of various human tissues, and that this combination is reversible for several hours, but subsequently becomes irreversible (3). This communication will describe the toxic effects of parathion in 32 men and 8 women following accidental exposure, and the relation of these effects to the depression of ChE enzymes. Recommendations are made for safeguarding against the absorption of parathion and for measures to be taken when such absorption occurs.

## NATURE OF EXPOSURE TO PARATHION

In the production of parathion for use as an insecticide the liquid parathion (97 per cent pure) is mixed with ataclay (a clay powder)

[1] The work described in this paper was carried out under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

[2] Trade names of the insecticide include: "Lethalaire G-54 Parathion Aerosol", "Chipman Parathion", "P.A.R. Parathion", "Phos Kit Parathion", "Paradust Parathion", "Dow Parathion", "Vapophos Parathion", "Penphos Parathion", "Aphamite Parathion", "Parathion Insecticides", "Genithion Parathion", "Edco 15 Parathion", "Niran (Parathion)", "Geigy Parathion", and "Thiophos (Parathion)".

to produce a clay dust with parathion adsorbed in concentrations varying from 15 to 25 per cent. This product is used as an insecticide in agriculture after dilution with clay to 1 to 2 per cent, or with water to 0.06 per cent parathion.

Parathion may be absorbed through the skin, respiratory tract, conjunctivae, gastrointestinal tract, or following injection. It is probable that the high solubility of parathion in lipoid media influences its absorption through the skin and its distribution in the body. It does not produce local inflammatory changes in the skin, so that absorption by this route may be undetected until symptoms begin.

Exposure to parathion has resulted in death in six[3] men, severe but not fatal symptoms in two men and two women, and mild to moderate symptoms in 24 men and six women. The nature of the exposure is summarized in Table 1. To our knowledge, symptoms have not yet occurred following the ingestion of fruit or vegetables that had been sprayed with this compound, but this remains a possibility, since fruit harvested from trees that had been sprayed with 0.06 per cent parathion nine days before harvesting contained up to 0.09 mg. per pound of fruit. Furthermore, mild to moderate symptoms attributable to parathion occurred in 20 subjects who harvested fruit from trees that had been sprayed with 0.06 per cent solution as long as nine days before harvesting (4).

The length of exposure to this compound prior to the occurrence of symptoms varied from several hours to several weeks, and no doubt depended on the degree of exposure and of absorption. All the men who died, and 27 of the men and women who had symptoms and recovered, had been exposed on one or more days during the month preceding the day on which symptoms occurred. The average period of exposure was 8 hours a day for 12 days. It is believed that some absorption probably preceded the last exposure in each instance, resulting in progressive inactivation of the ChE enzymes of the tissues and increased susceptibility to the action of parathion. In spite of this, only five of these subjects had any symptoms prior to the day on which severe complaints appeared. These warning symptoms, which appeared for one day to one week prior to the development of more severe manifestations, included one or more of the following: inter-

[3] See footnote to Table 1.

mittent nausea, vomiting, giddiness, weakness, drowsiness, and fasciculations of the eyelids. Unfortunately, these abnormalities were not reported, and exposure to parathion was continued. Seven of the men who had mild or moderate symptoms had had no known exposure to parathion prior to the day on which symptoms occurred.

One of the subjects who died, four who recovered after severe symptoms, and 18 after mild or moderate symptoms, had worn carbon filter respirators, rubber gauntlets and protective coveralls (which did not, however, completely cover the arms or neck) during their exposure to parathion. Some had also worn hip length rubber boots and a rubber apron. It is probable, however, that breaks in technique occurred, and one subject thought that her respirator was defective on the day that she developed severe symptoms. In addition, the facepiece respirators that were employed did not offer full protection against the inhalation of aerosol, dust, or spray.

Four subjects noted the onset of symptoms (nausea and vomiting) 10 to 30 minutes after eating. It is not clear whether this was due to the acceleration of intestinal absorption of parathion, or to the provocation of symptoms by the entry of food into the stomach.

### TIME INTERVAL BETWEEN EXPOSURE TO PARATHION AND ONSET OF SYMPTOMS

About half the subjects began to have symptoms while they were still exposed to parathion. The remaining subjects developed symptoms one half to eight hours (with an average of three hours) after their last exposure to parathion. Four of the six patients who died, and three of the four patients who survived after severe symptoms, walked into the hospital. One patient was admitted to the hospital in a comatose condition, and two died before they could be brought to a hospital.

### SIGNS AND SYMPTOMS DUE TO PARATHION

The signs and symptoms that followed exposure to parathion resembled in general those that followed the other organic phosphate antiChE compounds, di-isopropyl fluorophosphate (DFP) (5) and tetraethyl pyrophosphate (TEPP[4]) (6), and included muscarine-like,

[4] Trade names for TEPP insecticide include "Nifos-T" and "TEPP". Another organic phosphate antiChE compound, hexaethyl tetraphosphate (HETP), has

nicotine-like, and central nervous system effects (Table 1). The central nervous system manifestations due to parathion were more striking than those due to TEPP, and perhaps even DFP.

*Muscarine-like signs and symptoms:* The first symptoms to develop following exposure to parathion were usually anorexia and nausea. These were characteristically increased by smoking, and were usually soon followed by vomiting, abdominal cramps, excessive sweating and salivation. Most patients who had these symptoms also had some degree of pupillary constriction, but, when the symptoms were mild, this was minimal or absent. If the exposure was marked, diarrhea, tenesmus, involuntary defecation and urination, elevation of the blood pressure, pallor, pin-point non-reactive pupils, blurred vision, excessive bronchial secretion, and sometimes respiratory difficulty (suggestive of bronchoconstriction) and pulmonary edema with cyanosis followed. The severity of the respiratory manifestations was probably greatest when the respiratory tract was one of the routes of absorption. It is of interest that the blood pressure, instead of falling, as it may after the administration of neostigmine and of most direct acting cholinergic drugs, was frequently elevated, particularly in subjects with severe symptoms.

*Nicotine-like signs and symptoms:* Muscular fasciculations in the eyelids and tongue were early signs, usually occurring shortly after the nausea and vomiting. If the exposure was marked, this was followed by fasciculations in the muscles of the face and neck, in the extra-ocular muscles (resulting in jerking movements of the eyes, resembling nystagmus), and then by generalized fasciculations and weakness. Muscle cramps occurred in two subjects.

*Central nervous system signs and symptoms:* The earliest effects referable to the central nervous system were giddiness, uneasiness, restlessness, anxiety and tremulousness. These abnormalities usually occurred shortly after the onset of anorexia and nausea, but occasionally before. If the exposure was moderate these symptoms were followed by headache, sometimes by the sensation of "floating", and by insomnia, with excessive dreaming. Paresthesias, which occurred frequently after TEPP, and nightmares, which were common after DFP, were not often observed after parathion. If the exposure was marked,

also been used as an insecticide. Its effects are similar to those of TEPP, which is the chief active product of its initial hydrolysis.

TABLE 1

*Signs and symptoms that followed exposure to parathion*

| TYPE OF EXPOSURE | | NUMBER OF SUBJECTS WHO HAD SIGNS AND SYMPTOMS | | |
|---|---|---|---|---|
| | | Very severe | | Mild or moderate, 30 |
| | | Died, 6* | Survived, 4 | |
| Synthesis or handling of liquid parathion | | 2* | | 9 |
| Mixing liquid parathion with clay powder | | 1 | 2 | 5 |
| Packaging 15 or 25% parathion powder | | | 2 | 15 |
| Diluting 25% parathion powder and spraying field with 0.06 or 0.25% aqueous suspension of parathion or 2% parathion powder | | 3* | | 1 |
| Effector organ | Sign or Symptom | | | |
| 1. Muscarinic | | | | |
| a) Gastrointestinal | Anorexia, nausea | 6* | 4 | 20 |
| | Vomiting | 5* | 4 | 10 |
| | Abdominal cramps | 5* | 1 | 6 |
| | Diarrhea | 3* | 1 | 3 |
| | Tenesmus | 2* | 1 | 0 |
| | Involuntary defecation | 2* | 1 | 0 |
| | Eructation, "heartburn" | 1 | 1 | 2 |
| | Substernal pressure suggestive of cardiospasm | 1 | 1 | 2 |
| b) Sweat glands | Increased sweating | 5* | 4 | 14 |
| c) Salivary glands | Increased salivation | 3* | 2 | 0 |
| d) Lacrimal glands | Increased lacrimation | 2* | 2 | 0 |
| e) Heart and peripheral vascular | Increase in blood pressure | 3* | 4 | 0 |
| | Pallor | 4* | 3 | 6 |
| f) Lungs | Respiratory difficulty suggestive of bronchospasm | 2 | 2 | 5 |
| | Excessive bronchial secretion | 4* | 2 | 0 |
| | Pulmonary edema, with cyanosis | 3* | 1 | 0 |
| g) Pupils | Miosis (pin-point, nonreactive) | 5* | 3 | 4 |
| h) Ciliary body | Blurring of vision | 2* | 3 | 3 |

TABLE 1—*Continued*

| Effector organ | Sign or symptom | NUMBER OF SUBJECTS WHO HAD SIGNS AND SYMPTOMS | | |
|---|---|---|---|---|
| | | Very severe | | Mild or moderate, 30 |
| | | Died, 6* | Survived, 4 | |
| i) Bladder | Frequency | 2* | 2 | 0 |
| | Involuntary micturition | 1* | 2 | 0 |
| 2. Nicotinic | Muscular fasciculations: | | | |
| | eyelids, occ. tongue | 5* | 4 | 12 |
| | extra-ocular muscles | 5* | 4 | 4 |
| | generalized | 6* | 4 | 3 (mild) |
| | Muscle cramps | 1 | 0 | 1 |
| | Weakness | 5* | 4 | 6 (mild) |
| 3. Central Nervous System | Uneasiness, restlessness, anxiety, tremulousness | 6* | 4 | 8 |
| | Giddiness | 5 | 4 | 17 |
| | Headache | 3* | 2 | 12 |
| | Sensation of "floating" | 1* | 0 | 6 |
| | Insomnia, with excessive dreaming | 0 | 2 | 8 |
| | Ataxia | 3* | 2 | 0 |
| | Slurred, slow speech with repetition | 3* | 1 | 0 |
| | Drowsiness | 3* | 2 | 1 |
| | Difficulty concentrating | 3* | 2 | 1 |
| | Confusion | 2* | 2 | 1 |
| | Coma, with absence of reflexes | 6* | 2 | 0 |
| | Cheyne-Stokes respirations | 1* | 2 | 0 |
| | Convulsions | 5* | 2 | 0 |
| | Hyperpyrexia (to 107°F.) | 1 | 0 | 0 |

* Of the 6 subjects who died only one (A. N.) was under our personal observation. Signs and symptoms that were manifested by this patient are designated by an asterisk. We are indebted to Dr. Joseph Guelich, New York City, for the data on four fatal cases, and to Dr. D. O. Hamblin, Medical Director of the American Cyanamid Company, for the data on one fatal case (K. H.) and for permission to examine the tissues of this patient. The four patients who survived after severe symptoms, and the 30 who had mild or moderate effects, were under our personal observation.

ataxia, tremor, drowsiness, difficulty in concentrating, mental confusion, occasionally disorientation, and changes in speech developed. The speech difficulties were characterized by slurring, difficulty in

forming words and in self-expression, and multiple repetition of the last syllable. This was followed, in the most severe cases, by the gradual development of coma. The six patients who died, and two who recovered, went into profound coma, with the disappearance of all reflexes. The coma began an average of 4 hours after the onset of symptoms (range 1–9), and the average duration of the coma was 3½ hours (range ¼–9). Three of the six patients who died regained consciousness prior to exitus. In most instances the recovery of consciousness appeared to follow the administration of atropine. Two of the three patients who remained comatose were given no atropine. Following the return of consciousness the administration of atropine was unfortunately diminished or stopped. In the three patients in whom the restored consciousness was temporary, this lasted an average of 3½ hours, following which the coma returned and death followed.

Six of the eight patients who became comatose had repeated generalized convulsions after the onset of coma. One of the subjects who died and two who survived had Cheyne-Stokes respiration during part of the time they were comatose. Because of the pin-point pupils that were present in all the subjects who had severe symptoms, an adequate examination of the optic nerve head was performed in only one of these subjects, after the instillation into one eye of one per cent atropine every 15 minutes over a period of one hour. The atropine produced incomplete dilatation of the pupil, and enabled the detection of papilledema which measured about two diopters. One other patient had a lumbar puncture performed prior to death which revealed the spinal fluid to be under increased pressure, but otherwise normal.

*Blood pressure:* The blood pressure was elevated in most patients who had moderate or severe symptoms, and was not depressed except shortly before death. Two of the patients who died, and the four who survived after severe symptoms, were known to have been normotensive prior to the onset of symptoms. On admission to the hospital, at which time five of these patients were having moderate, and one severe symptoms, their average blood pressure was 164/100, and in one patient the blood pressure was 200/130. There was no evident anoxia to which this elevation of the blood pressure might be attributed. As the symptoms became more severe there was usually a further increase in blood pressure. When signs of improvement ap-

peared, even though only transiently in the two patients who later died, there was in each instance a fall in the blood pressure to normal levels, to an average of 130/75.

*Body temperature:* One of the patients who died had hyperthermia (107°F.) just before death. Another, who survived after severe symptoms, including pulmonary edema, had some elevation of temperature (to 101°F.) on the first, second, and third days after exposure to parathion. In the remaining patients no significant abnormality of body temperature was noted.

*Laboratory data:* A number of laboratory tests were performed in the four patients who recovered after severe symptoms, and in one of the patients who died. No appreciable abnormality was found in the following: red blood count, hematocrit, hemoglobin, sedimentation rate, urinalysis, blood non-protein nitrogen and urea nitrogen, serum carbon dioxide combining power, serum amylase, cephalin flocculation, alkaline phosphatase, total protein, and albumin-globulin partition. No alteration of the electrocardiogram was detected. In one patient the stool was strongly positive for occult blood while diarrhea was present, but not later.

In 12 patients in whom the leucocyte count was determined there was a slight to moderate leucocytosis in every instance, the average white blood count being 15,000 and the range 13,000 to 20,000. The differential white blood count revealed a slight increase in the per cent of mature polymorphonuclear leucocytes, the average differential count being 2 per cent juvenile and 86 per cent segmented leucocytes, and 12 per cent lymphocytes.

*Treatment that was administered:* Atropine was administered to four of the six men who died, and to all four subjects who had severe symptoma and who survived. The average total amount administered (hypodermically) was 2 mg. Although this amount is considerably below what is now considered optimal, it resulted in moderate diminution of the salivation, sweating, bronchial secretion, respiratory and gastrointestinal symptoms, and less marked improvement in the central nervous system manifestations. In some subjects the miosis improved slightly over a period of one or more hours. There was no change in the muscular fasciculations or weakness following the administration of atropine.

One of the men who subsequently died received curare (140 units intravenously over 3 minutes, followed in ½ hour by 100 units intravenously) for the purpose of diminishing the severity of the convulsions. Another, who survived, received 200 units of curare intravenously. The administration of curare resulted in the immediate, though temporary, cessation of muscular fasciculations, and temporary diminution in the severity of the convulsions. Since there was no respiratory depression after the administration of these large doses of curare, it is very probable that there was increased tolerance to this drug, attributable to the antiChE action of parathion. Another patient who later died received sodium nembutal intravenously, with temporary diminution in the severity of the convulsions. Other adjuvants to therapy included the administration of oxygen (with 5 per cent carbon dioxide when Cheyne-Stokes respiration was present), and the intravenous administration of saline, glucose, and plasma.

*Duration of symptoms in patients who survived:* The average duration of the acute effects in the four patients who had severe symptoms and who survived was 25 hours (range 18–30), and in the 30 patients who had less severe symptoms 12 hours (range 6–24). There were also effects of parathion intoxication which were more prolonged. All four patients who recovered after severe illness had mild anorexia, giddiness (especially on getting out of bed), uneasiness, anxiety, insomnia, headache, and weakness for 48 to 72 hours after their last exposure to parathion. One patient had mild weakness and abnormal fatigability for a week. Two patients complained of moderate tension, anxiety, depression (with crying spells), insomnia, and mild anorexia for periods of one and three weeks after their last exposure to parathion.

The pin-point pupils which were present in patients whose symptoms were moderately severe usually returned to normal size slowly over a period of 36 hours. In two patients, however, the time required for the pupils to return to normal size was three weeks, and in one patient six weeks. It is probable that these latter three patients had sprayed or rubbed parathion into their eyes. This is suggested by differences in the rate of disappearance of the miosis in the two eyes, resulting in marked pupillary inequality, and by the persistence of miosis in one patient after the red blood cell ChE had returned to normal activity.

There was no detectable sex or age difference (age limits 23 to 58) in

the incidence, severity or duration of symptoms of parathion intoxication. This is in contrast to the difference in susceptibility between the sexes which has been described in mice and rats, with females being more susceptible (1).

*Time of occurrence and description of death:* The average time interval between the last exposure to parathion and death was 10½ hours (range 1–21½), and between the onset of symptoms and death 9 hours (range 1–13½). The immediate cause of death is not known. The sequence of terminal events appeared to be the following: the respirations became shallow, labored, and rapid, and in some instances there were râles and cyanosis; then, the blood pressure fell, became unobtainable, and within 15 to 30 minutes death ensued. Contributing factors may have been depression of the respiratory and circulatory centers in the medulla, weakness of the muscles of respiration, and pulmonary edema.

*Post-mortem findings:* Post-mortem examination was performed in five of the six patients who died. In four there was capillary dilatation, hyperemia, and edema of the lungs; in two, of the brain as well; and in one, of all the organs, including the lungs, liver, spleen, kidneys, and brain (7). In the two patients in whom cerebral edema was noted, there was an increased amount of clear cerebrospinal fluid in the ventricles and subarachnoid space, as well as a "pressure cone". In one patient there was some pneumonitis, but this was not considered to be sufficient to account for death. The organs of two patients were examined for parathion, which was found in the blood, brain, liver, and kidneys in concentrations of 0.11 to 0.17 mg. per 100 grams of tissue in one patient (7), and 0.01 to 0.11 mg. per 100 grams in the other.

### ANTICHOLINESTERASE ACTION OF PARATHION IN VIVO

*Effect on plasma and red blood cell ChE activity:* Four patients who survived despite severe symptoms, and two who died, had marked depression of the ChE activity of the plasma to 5 per cent of normal activity or less, and of the red blood cells to 11 to 22 (average 14) per cent of normal activity. Six other subjects with symptoms of moderate degree had slightly less marked depression of the ChE activity of the plasma to an average of 10 per cent of normal activity, and of the red blood cells to 12 to 28 (average 22) per cent of normal activity.

*Effect on tissue ChE activity:* The ChE activity of the tissues of two of the patients who died following exposure to parathion was determined post-mortem (Table 2). One patient, a 35 year old white man (A. N.) who had had repeated exposure to liquid parathion and to parathion adsorbed on ataclay (15 to 34 per cent), developed marked cholinergic symptoms half an hour after his last exposure, became comatose 2½ hours later, and died 10 hours after the onset of symptoms.

TABLE 2

*Comparison of the ChE activity of various tissues of two subjects (A. N. and K. H.) who died after exposure to parathion with the average ChE activity of 8 subjects who had received no exposure to any antiChE agent and who had no disease of the central nervous system, liver, kidney, or blood forming organs.*

The control average ChE activity is expressed in millimoles of acetylcholine bromide hydrolyzed per minute per gram of tissue per ml. Determinations were made manometrically (5).

| | CHOLINESTERASE ACTIVITY | | |
|---|---|---|---|
| | Control average (mM ACh Br × $10^{-4}$) | Per cent of control average | |
| | | A. N. | K. H. |
| Plasma | 9.9 | 5 | 0 |
| Red blood cells | 14.0 | 11 | 22 |
| Liver | 3.7 | 40 | 80 |
| Kidney | 1.0 | 88 | |
| Cerebral cortex | 2.9 | 22 | 31 |
| Thalamus | 22.5 | 54 | 50 |
| Cerebellum | 14.8 | 55 | 50 |
| Pons | 6.0 | 39 | 43 |
| Medulla | 3.1 | 38 | 40 |

The clinical course of this patient has been described in detail in a previous report (7). The other patient (K. H.), a 31 year old white male who had been exposed repeatedly to 25 per cent parathion adsorbed on ataclay and to 0.06 per cent aqueous parathion spray, developed marked cholinergic symptoms during his last exposure and died one hour later. The ChE activity of the plasma and red blood cells of patient A. N. was markedly depressed to 5 and 11 per cent, and of patient K. H. to 0 and 22 per cent of normal activity. The ChE activity of various parts of the brain, and of the liver and kidney of both patients was also depressed, but, except for the cerebral cortex,

the diminution was not of the same degree as that of the plasma and red blood cells, the activity being 38 to 88 per cent of the average "normal".

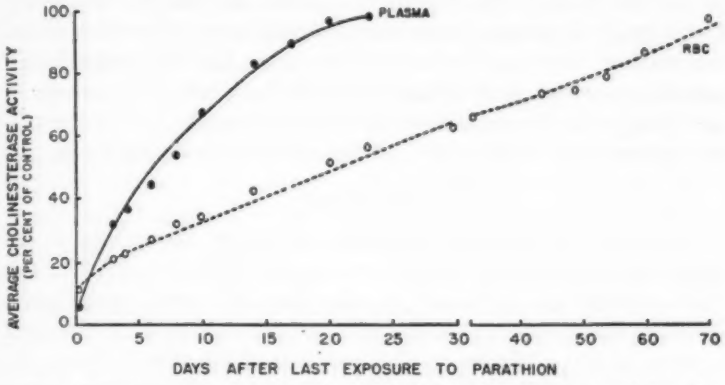


FIG. 1. Recovery of plasma and red blood cell cholinesterase activity after cessation of exposure to parathion. (Average values obtained in 18 subjects.)

TABLE 3

*The Rates of Return of Plasma and Red Blood Cell Cholinesterase Activity in Man after Depression by Parathion, and Comparison with DFP and TEPP. (Average values obtained in 18 subjects after parathion, 35 subjects after DFP, and 14 subjects after TEPP.*

| DAYS AFTER CESSATION OF ADMINISTRATION OR EXPOSURE | AVG. RATE OF RETURN OF ChE ACTIVITY PER DAY (IN PER CENT OF NORMAL ACTIVITY) | | | | | |
|---|---|---|---|---|---|---|
| | Plasma ChE | | | RBC ChE | | |
| | DFP | Parath. | TEPP | DFP | Parath. | TEPP |
| 1–3 | 6 | 9 | 13 | 1.3 | 3.3 | 6 |
| 4–10 | 5 | 5 | 5 | 1 to 2 | 1 to 2 | 1 to 2 |
| 10–20 | 3 | 3 | 3 | 1 to 2 | 1 to 2 | 1 to 2 |
| 20–30 | | | | 1 to 2 | 1 to 2 | 1 to 2 |

## RETURN OF PLASMA AND RED BLOOD CELL ChE ACTIVITY IN VIVO AFTER DEPRESSION BY PARATHION (FIG. 1 AND TABLE 3)

When 18 subjects whose plasma and red blood cell ChE activity had been depressed by parathion were removed from further exposure, the plasma ChE increased at an average rate of approximately 27 per cent of normal activity during the first three days. This rate of return fell

off to 5 per cent per day by the fourth day and 3 per cent by the tenth day. The red blood cell ChE increased at an average rate of approximately 10 per cent of normal activity during the first three days. This rate of return diminished to between 1 and 2 per cent per day by the fourth day, and remained fairly constant until a normal level of activity was reached. The rate of return of both plasma and red blood cell ChE activity appeared to be independent of the degree of exposure to parathion or the degree of depression of ChE activity, and, if the subject survived, the ChE activity always returned to normal levels.

## DISCUSSION

The effects of parathion resembled in general the effects of the other organic phosphate antiChE compounds, DFP (5) and TEPP (6). These included muscarine-like, nicotine-like, and central nervous system signs and symptoms which could, for the most part, be explained in terms of the inhibition of ChE enzymes of the effector tissues by each compound. The muscarine-like effects of each compound were inhibited by atropine, and one of the nicotine-like effects (muscular fasciculations) was inhibited by curare. In addition, the tolerance for atropine and curare was increased in patients who had symptoms due to any of these compounds.

Most of the observed effects of parathion are compatible with its antiChE action. Correlation of these effects with the physical and chemical (antiChE) properties of parathion (3) lends support to this being the predominant, though probably not the only mode of action of this compound.

*Time of onset of symptoms due to the antiChE compounds:* The time interval between exposure to parathion and the onset of symptoms was in general somewhat longer than that seen following DFP, TEPP, or neostigmine. This difference may possibly be correlated with the somewhat slower rate of inactivation of ChE enzymes in vitro by parathion than by DFP, TEPP, or neostigmine.

*Duration of symptoms and of cumulative effects of the antiChE compounds:* The acute symptoms that followed exposure to parathion lasted 6 to 30 hours, and the severity of these symptoms was cumulative following exposure at intervals of one to several days. In this regard parathion resembled TEPP and was intermediate between DFP and

neostigmine. The acute symptoms produced by DFP were of longer duration (up to 96 hours), and the cumulative effects of this compound were more striking and occurred over a longer interval between doses (up to 3 weeks). The acute symptoms produced by neostigmine are of much shorter duration (less than four hours), and there are no cumulative effects after this time.

There is a fairly good correlation between the duration of symptoms and cumulative effects of these antiChE compounds, and the reversibility of the ChE-antiChE combinations in vitro. The combination of DFP with ChE is completely irreversible after the first few minutes of combination, so that, in vivo, ChE activity is probably restored only as a result of the regeneration of new enzyme protein, which occurs slowly over a period of weeks. In contrast, the combination of neostigmine with ChE is completely reversible, and, in the body, neostigmine is completely removed (detoxified and excreted) within a few hours. The combination of parathion or TEPP with ChE appears to be partly reversible during approximately the first 24 hours of their reaction, and irreversible after that time. It is probable that, following the inactivation of ChE by parathion or TEPP in vivo, part of the ChE activity is restored within 24 hours as a result of partial dissociation of the ChE-antiChE combination, and that, following this period, ChE reappears only as a result of the regeneration of new enzyme protein.

*Central nervous system effects of the antiChE compounds:* The central nervous system effects of parathion were striking. In contrast, neostigmine has very little effect on the central nervous system (5). TEPP has much more effect on the central nervous system than neostigmine, but less than DFP or parathion. The central nervous system effects of DFP are marked, and approach those of parathion. There is a reasonably good correlation between the solubility of these antiChE compounds in peanut oil, as compared to water, and the degree of their central nervous system effects. It is possible that the high lipoid content of the central nervous system may result in the ChE enzymes of the central nervous system being more accessible to antiChE agents which are more soluble in lipoid than in aqueous media. The affinity of parathion for peanut oil as compared to water was much higher than that of neostigmine or TEPP, and slightly higher than that of DFP.

*Relation of effects of the antiChE compounds to the ChE activity of the tissues:* Observations following the administration of DFP and of TEPP to human subjects indicate that these compounds exert their effects by depressing the ChE activity of the tissues below a threshold at which changes in function begin. The difference between this threshold and the level of ChE activity below which serious and even fatal functional alterations occur appears to be narrow. There is evidence which suggests that symptoms due to DFP or TEPP begin when the ChE activity of the effector organs is reduced to somewhere between 25 and 50 per cent of normal activity, and that death occurs when the ChE activity of the brain is reduced by these compounds to below approximately 20 per cent of normal activity (8).

The ChE activity of the tissues of two patients who died following exposure to parathion was moderately depressed, but not to the same degree as occurred in patients who died following DFP (8). Death due to parathion appears to have occurred at a significantly higher level of ChE activity of the brain than did death due to DFP. Since there is convincing evidence that DFP acts solely by virtue of its antiChE activity, several explanations of this difference are possible:

1. The ChE activity of a vital center in the brain may have been depressed to a greater extent by parathion than was reflected by the ChE activity of the larger segments which were examined.

2. The ChE activity of the brain may have been transiently depressed by parathion below the level at which serious functional alterations occurred. Then, following removal from further exposure to parathion, death may have ensued despite some restoration of ChE activity. It is of interest that three of the six patients who died following exposure to parathion showed transient clinical improvement prior to death. In experimental animals delayed death due to parathion has been described, after an initial partial improvement and some restoration of ChE activity of the tissues (1).

3. The respiratory tract may have been one of the routes of absorption of parathion, resulting in the respiratory manifestations being out of proportion to the other effects.

4. Parathion may have some pharmacologic effect in addition to that produced by its antiChE action. Moderate elevation of blood pressure, leucocytosis, and post-mortem cerebral edema and hyperemia,

which occurred following parathion, were not observed in man following DFP or TEPP. The administration of atropine to experimental animals prior to parathion has a definite protective effect (1), but this appears to be quantitatively less striking than the protective effect of atropine against DFP (9) or TEPP (10).

*Relation of effects of the antiChE compounds to the ChE activity of the plasma and red blood cells:* The cholinergic effects of antiChE compounds are due to the depression of the activity of the ChE enzymes of the nervous system, muscle, and secretory glands, and not of the plasma or red blood cells. However, since it is not possible to determine the ChE activity of these tissues in man during life, it is necessary to rely on the ChE activity of the plasma and red blood cells as a guide of some value in detecting absorption of the antiChE compounds. The ChE enzyme of the plasma was more sensitive to inhibition by parathion, DFP, or TEPP in vitro than were the ChE enzymes of the red blood cells, brain, and muscle, which were approximately equally sensitive to inhibition by each antiChE compound (3). As would be expected, a limited exposure to any of the antiChE compounds resulted in reduction of the ChE activity of the plasma before that of the red blood cells and before the development of any symptoms. Therefore depression of the ChE activity of the plasma was the first indication of absorption of any of the antiChE compounds, but could not be used as a guide to the ChE activity of the tissues. The ChE enzyme of the red blood cells, on the other hand, being as sensitive to inhibition by these compounds as were the ChE enzymes of the tissues, could be used as a rough guide to the latter if the exposure to the antiChE compound was relatively brief. If the exposure was over a longer period of time, this guide was less reliable, since the rate of restoration of the ChE activity of the red blood cells is probably slower than that of the tissues. Thus, the activity of the red blood cells could be gradually depressed toward zero by several days of exposure without symptoms necessarily ensuing, or without any relation to the severity of symptoms that occurred. In addition, the red blood cell ChE remained at a low level of activity long after the disappearance of symptoms and the presumed restoration of the ChE activity of the tissues. Finally, it is possible that absorption of parathion through the respiratory tract may produce local manifestations which are out of proportion to the

systemic effects, including the depression of plasma and red blood cell ChE activity.

After depression by parathion, the ChE activity of the plasma and red blood cells (Table 3) and of the tissues (1) returned more rapidly during the first day or two than after depression by DFP, but not quite as rapidly as after depression by TEPP (Table 3). The former difference is probably due to the partial reversibility of the ChE-parathion combination, in contrast to DFP, and the latter difference to the slower rate of hydrolysis of parathion, which would tend to retard the dissociation of this combination. Parathion is more rapidly hydrolyzed in vivo than in vitro, probably by an enzyme resembling the tissue enzyme (called phosphofluorase (11)) which accelerates the hydrolysis of DFP, but the rate of hydrolysis of parathion in vivo appears to be slower than that of DFP or TEPP. It is possible that differences may also exist in the rate of removal of each antiChE compound by excretion, combination with proteins other than ChE, and other means of detoxification.

After the initial period of one or two days, the rate of return of plasma and red blood cell ChE was approximately the same after depression by parathion as after DFP or TEPP, and was compatible with the regeneration of new enzyme protein by the liver (for plasma ChE) and in the red blood cells.

*Effects of parathion which may not be directly due to its antiChE action:* The moderate elevation of blood pressure, leucocytosis, cerebral edema and hyperemia, and hyperemia of the other organs, which were observed following exposure to parathion, were not seen following the administration of DFP or TEPP to man. The mechanism of these effects of parathion is not entirely clear.

Elevation of blood pressure due to an antiChE compound has been observed in man only after parathion, but in experimental animals it has been noted after TEPP as well (1, 12). This elevation of blood pressure may be due to cholinergic stimulation of the preganglionic innervation of the adrenal medulla (resulting in the release of adrenalin), to cholinergic stimulation of the sympathetic ganglia (resulting in splanchnic or generalized vasoconstriction), or to a direct stimulating action on the vasoconstrictor fibers of the arterioles. The occurrence of leucocytosis after exposure to parathion is compatible with the release of adrenalin.

The cause of the terminal fall in blood pressure after exposure to lethal amounts of parathion, DFP, or TEPP is not known. It is probably predominantly central in origin, though a direct depressive action on the heart and blood vessels has not been excluded.

*Some considerations concerning the lethal doses of the antiChE compounds:* The lethal doses of the organic phosphate antiChE compounds are not known for man. The $LD_{50}$ of parathion in rats and mice is approximately equal to that of DFP, and is ten times that of TEPP (3). The toxicity of parathion in these animals is greater than would be expected from its antiChE activity in vitro (3), and may be related to the marked solubility of parathion in lipoid media, with resulting greater affinity for the central nervous system, or perhaps to some

TABLE 4

*Single Doses of TEPP and DFP that Produced Moderately Severe Symptoms in Man, and Single Doses that are Estimated* to be Near the Minimal Lethal Dose*

| | SINGLE DOSE (MG.) REQUIRED TO PRODUCE MODERATELY SEVERE SYMPTOMS | | | SINGLE DOSE (MG.) THAT WOULD PROBABLY BE LETHAL | | |
|---|---|---|---|---|---|---|
| | Route of admin. | | | | | |
| | I.M. | Oral | Cutaneous | I.M. | Oral | Cutaneous |
| TEPP | 5 | 25 | ? | 20* | 100* | ? |
| DFP | 5 | 25 | 100*(= 0.1 cc.) | 25* | 125* | 500*(= 0.5 cc.) |

additional pharmacologic effect. Another possibility is that parathion may be oxidized to some extent in vivo to the oxygen analogue (substitution of O for S), which is 300 times more potent as an antiChE agent in vitro and about five times as toxic to experimental animals (1).

In Table 4 are recorded *estimations* of the lethal doses of DFP and TEPP in man. It is, of course, impossible to predict accurately the dose-effect relationship in one species from data obtained in another species. However, since the toxicity of parathion and DFP for experimental animals is comparable, and since their effects in man are in general similar, it is possible that the dose-effect relationship of parathion for man may approximate that of DFP.

*Hazards in handling the organic phosphate antiChE compounds:* These compounds can be absorbed by any route, including the skin. Their effects are prolonged and cumulative. Until the ChE enzymes of the tissues have been restored, which apparently requires many days,

subjects who have been exposed to these compounds have increased susceptibility to their effects. Since there is a fairly narrow margin between the doses of these compounds that produce symptoms and the doses that are lethal, little or no warning may be given of impending serious effects. It is probable that the ChE activity of the tissues may be considerably reduced without the appearance of any warning symptoms, while a further reduction below the level compatible with normal function may result in marked symptoms and even death.

Although parathion has lower antiChE activity and toxicity than TEPP, there are two, or perhaps three factors which are responsible for the greater efficiency of parathion as an insecticide. Unfortunately these same factors are responsible for the greater danger to man and domestic animals associated with the use of parathion. First, and most important, the rate of hydrolysis of parathion is very much slower than that of TEPP, 120 days being required for 50 per cent hydrolysis of parathion at 25°C. and pH 7. Hence, once parathion has been sprayed on trees or on fields, it remains active for weeks in spite of contact with moisture, in contrast to TEPP, which hydrolyzes within several hours. Second, parathion is very much more soluble in lipoid than in aqueous media, in contrast to the greater solubility of TEPP in aqueous media. Therefore, it would be expected to accumulate in the waxy outer layer of fruit and leaves, and has been found there as long as 9 days after spraying (13). A third factor is the considerably greater antiChE activity and toxicity of the oxygen anologue of parathion (1). It is known that this analogue may occur as an impurity in the manufacture of parathion, but it is not yet known to what extent oxidation to the more potent oxygen analogue may occur when parathion is exposed to the air, as in spraying, or in plant tissues, or after it is absorbed into the body.

Because of its more widespread use as an insecticide, and because of its greater persistence, parathion has been responsible for more instances of toxic exposure than has TEPP. However, the dangers and problems associated with the production, handling, and use of these compounds are quite similar, and there has been one report of serious symptoms following exposure to TEPP during its use as an insecticide (14). In addition, serious effects have occurred following the administration of overdoses of TEPP during the treatment of patients with

myasthenia gravis (6). The availability of insecticide preparations of TEPP and of parathion has also led to their ingestion for the purpose of suicide.

*Precautionary measures recommended for personnel exposed to the anti-ChE compounds:* The following safety measures to reduce the degree of exposure and of absorption are recommended for any situation in which there is exposure to parathion, or to any other organic phosphate antiChE compound:

1. Adequate warning labels, and distribution only to properly instructed personnel.

2. *To reduce absorption through the skin* complete protective clothing, including rubber gloves and goggles, should be worn and changed daily. Personnel should take a shower at the end of each day's work.

3. *To reduce absorption through the respiratory tract* respirators should be worn. If indoors, there should be adequate exhaust ventilation. If outdoors, dispersal of the antiChE compound by wind to unprotected personnel or domestic animals should be avoided.

4. *To reduce absorption through the gastrointestinal tract* employees should change clothing and wash before eating or smoking, and all food should be protected from exposure. Fruit, vegetables or tobacco should be sprayed only with very dilute solutions, harvested not less than several weeks after the last spraying, and thoroughly washed prior to use.

5. *Personnel contaminated with concentrated antiChE compound* should have clothes removed immediately and the skin washed with soap and water. They should be observed for at least 8 hours before going home. In case of serious contamination atropine may be administered prophylactically, in doses of one to two mg. parenterally. If this is done the subject should be carefully observed for at least 24 hours, lest the onset of serious symptoms be delayed by the administration of atropine.

6. *Material contaminated with concentrated antiChE compound* should be thoroughly washed with as concentrated a solution of alkali as is practicable, or burned. Preparations of the antiChE compounds should be carefully disposed of, with due consideration to their public health hazard.

7. *To prevent cumulation of the effects of the antiChE compounds,*

which may result in greatly increased susceptibility, personnel who are exposed frequently should have periodic determinations of the ChE activity of the plasma and red blood cells, or at least of the plasma. A simple method for the determination of ChE activity is available which can be employed with a minimum of equipment and of technical help (15). Any personnel who develop reduction in ChE activity should be removed from all exposure to any organic phosphate antiChE agent until the ChE activity has returned to normal, which will usually require several weeks.

The above safety measures were introduced with considerable success in one chemical company in which several instances of serious and even fatal parathion intoxication had previously occurred.

*The treatment of symptoms due to the antiChE compounds:* The mainstay is atropine, which has a moderate inhibitory effect on the muscarinic, and a less striking effect on the central nervous system manifestations. Patients who have moderately severe symptoms due to the antiChE compounds have an increased tolerance for atropine, so that fairly large doses may be given. It is recommended that 2 mg. of atropine be administered intramuscularly at hourly intervals, or more often if necessary, until signs of atropinization appear. Following this, the dose of atropine may be reduced, but its administration should be continued as long as any muscarine-like manifestations of the antiChE compound are present.

Adjuvants to atropine therapy include washing the skin and gastric lavage to remove any unabsorbed antiChE agent, parenteral replacement of fluids, and the administration of oxygen if needed. If pulmonary edema is not relieved by atropine, the careful administration of oxygen under pressure may be of value. Morphine should not be administered, as its action may be potentiated by antiChE compounds. The occurrence of marked weakness of the pharyngeal muscles, or the appearance of laryngospasm accompanying fasciculation of the laryngeal muscles, may require tracheal intubation. If marked weakness of the muscles of respiration appears, artificial respiration may be necessary. The administration of curare abolishes muscular fasciculations and diminishes the severity of convulsions, but, since an overdose may cause weakness of the muscles of respiration, its use is probably not advisable. If the convulsions are very severe, the

careful administration of ether or of a barbiturate for their amelioration may be of value.

## SUMMARY

1. Parathion (p-nitrophenyl diethyl thionophosphate) may be absorbed through the skin, respiratory tract, gastrointestinal tract, or following injection. Accidental exposure to this compound (usually repeated) during its preparation or use as an insecticide has resulted in death in six subjects, severe but not fatal symptoms in four, and mild to moderate symptoms in thirty.

2. Manifestations that followed exposure to parathion included muscarine-like, nicotine-like, and central nervous system effects, resembling those produced by the other organic phosphate anticholinesterase (antiChE) compounds, di-isopropyl fluorophosphate (DFP) and tetraethyl pyrophosphate (TEPP). Death occurred in coma 18 to 30 hours after the onset of symptoms, and usually after the occurrence of generalized convulsions.

3. Parathion produced a marked depression of the ChE activity of the plasma and red blood cells in all subjects examined, and a moderate depression of the ChE activity of the tissues (including the brain) in the patients who died. Following cessation of exposure to parathion the plasma and red blood cell ChE activities were restored at rates which, after the first three days, were compatible with the regeneration of new enzyme protein by the liver and by the red blood cells.

4. Most of the effects of parathion, and almost all the effects of DFP and TEPP, appear to be due to the depression of the activity of the ChE enzymes of the tissues. Differences between the clinical effects of these compounds can for the most part be explained by differences in their physical and chemical properties. The elevation of blood pressure and leucocyte count produced by parathion, and the post-mortem finding of hyperemia and edema of the organs, suggest that this compound may have some additional pharmacologic effect.

5. Because of its high lipoid solubility and very slow rate of hydrolysis, the handling of parathion is especially hazardous. Recommendations are made for the safety of personnel exposed to this, and other antiChE compounds.

6. The treatment of symptoms due to the organic phosphate anti-

ChE compounds relies chiefly on atropine, which may be administered in large doses. Supplementary measures include the removal of unabsorbed compound, parenteral replacement of fluids, the administration of oxygen, and, if necessary, tracheal intubation and artificial respiration.

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# THE DETERMINATION AND SIGNIFICANCE OF SUBSTANCES NEUTRALIZING NEWCASTLE VIRUS IN HUMAN SERUM[1]

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## INTRODUCTION

Newcastle Disease is a widespread common virus disease of chickens. Both abroad and in this country the virus has been recovered from cases of mild conjunctivitis occurring in laboratory workers and in veterinarians exposed to infected fowl (1, 2, 3, 4). It may also be a fairly common cause of conjunctivitis in poultry workers who handle infected flocks, one small outbreak of this kind having been reported in Palestine (5). Recovery of Newcastle Disease virus from such cases incriminates it as the probable agent, although neutralization tests so far have failed to demonstrate significant antibody in the affected patient (3).

With the finding that people who have not had conjunctivitis may, nonetheless, have a high titer of "neutralizing" substance in their sera (6), the question of the extent of infection of man by this virus was raised. To answer this, it is necessary to know how many people have these neutralizing substances and under what conditions an infection will evoke them.

It has been known for some time that various sera contain heat labile and heat stable substances, presumably "non-specific", which will neutralize various viruses (7). It has been recently demonstrated by several workers (8, 9, 10, 11) that there is a heat labile substance present in human sera which neutralizes several viruses, including that of Newcastle Disease. The presence of this substance means that the usual testing of paired sera from a patient with an acute infectious disease must be done advisedly. If the tests are run simultaneously,

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the first serum, drawn early in the disease, may have lost the substance, while the second, more recently obtained, may still contain it. The second serum neutralizes the virus whereas the first does not, leading to the false premise that the patient is infected with, or has had an antibody response to Newcastle virus (12).

Understanding of this heat labile substance is further confused by another factor. It has been observed in several reports (13, 14, 15) that a heat labile component of serum, presumably complement, improves and assists in the neutralization test against certain viruses; that precautions should therefore be taken to preserve its function.

Such conflicting evidence emphasizes the need of a careful evaluation of the neutralization test, using one virus on one kind of test animal in a series of experiments. We here report on some factors which influence the neutralization of Newcastle Disease virus; the effects of heated and unheated human sera are compared with the effect of Newcastle-convalescent chicken sera. The combination of virus and serum is inoculated on chick embryos. One heated human serum, which consistently neutralized Newcastle virus, is compared with heated rabbit sera.

## METHODS

Strain "B" of Newcastle Disease virus was used throughout (16), with the exception of one experiment mentioned in Table II. The virus was kept frozen as freshly harvested allantoic fluid in a $CO_2$ deep freeze unit in celluloid tubes. Individual tubes were used in each test and were then discarded. New batches of fresh virus were prepared every few months. All crucial tests were paralleled by controls in which the activity of the diluted virus was determined following simultaneous incubation for one hour at 37°C. in an equal volume of normal heated (56°C., 30 min.) chicken sera. Incubation of virus with such heated chicken sera for this period of time does not cause a measurable loss of activity.

Following incubation, the combinations of virus and sera were inoculated on the chorio-allantoic membrane of eleven day old chick embryos. These were checked for mortality during the next six days. The 50 per cent endpoint of the control and the test sera were calculated from these results (17), and the difference expressed as the amount of virus neutralized. We have in many cases presented the complete experimental results instead of merely the calculated endpoint, for an excessive variation in mortality at different dilutions of virus might alter the calculated endpoint without representing actual neutralization of virus.

Immune chicken sera were obtained from chickens recovering following infection with about 10,000 embryo lethal doses of the "B" strain of virus. Immune

rabbit sera were prepared by injecting a rabbit with 1 cc. of washed 10X concentrated virus, which would titer to about $10^{-9}$ (16). The allantoic fluid virus is obtained by inoculating eleven day old embryos in the allantoic sac and incubating for 48 hours at 35°C.

Complement was specifically removed from fresh human serum by the addition of an antigen (egg albumin) and an equivalent quantity of the corresponding antibody (rabbit anti-egg albumin) (18).[3] The mixture was held at 4°C. over-

TABLE I

*The effect of heating various normal human sera on the neutralizing activity of these sera against Newcastle virus in the embryo*

| DILUTION | F. I. | | CONTROL NORMAL HEATED CHICKEN SERUM | D. K. | | I. K. | | J. S. M. | | CONTROL NORMAL HEATED CHICKEN SERUM |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unheated | Heated | | Unheated | Heated | Unheated | Heated | Unheated | Heated | |
| $10^{-2}$ | | | 0/5 | | | | | | | |
| $10^{-3}$ | | | 4/5 | | | | | | | 1/4 |
| $10^{-4}$ | 1/5* | 4/4 | 3/5 | 0/5 | 3/3 | 1/4 | 3/5 | 0/4 | 0/4 | 4/4 |
| $10^{-5}$ | 0/5 | 5/5 | | 2/5 | 5/5 | 0/4 | 5/5 | 0/4 | 3/4 | |
| $10^{-6}$ | 1/5 | 5/5 | | 1/5 | 5/5 | 0/3 | 5/5 | 0/4 | 4/4 | |
| $10^{-7}$ | 4/5 | 5/5 | | 1/5 | 5/5 | 0/4 | 5/5 | 0/4 | 4/4 | |
| $10^{-8}$ | | | | | | | | | | |
| LD 50 of combination.... | 4.7 | 7.5+ | 7.8 | 4.0 | 7.5+ | 3.5− | 6.8 | 3.5− | 6.3 | 7.7 |
| LD 50 neutralized.... | 3.1 | <0.3 | — | 3.7 | <0.2 | >4.2 | 0.9 | >4.2 | 1.4 | — |

* Enumerator—Number dying from Newcastle virus within six days.
Denominator—Number of embryos inoculated.

night, after which the precipitate was separated by centrifugation. For every cubic centimeter of serum to be decomplemented, 0.20 mg. of antibody nitrogen and 0.02 mg. of egg albumin nitrogen were added. This system was effective in producing more than a 20 fold reduction of the complement activity of serum as measured by hemolysis of a 5 per cent suspension of sensitized sheep RBC.

## RESULTS

In Table I it may be seen that all four of the fresh, undiluted human sera, when combined with various dilutions of Newcastle virus

[3] Supplied through the kindness of Dr. Manfred Mayer.

and incubated at 35°C. for one hour, neutralized 10,000 LD 50's of Newcastle virus. All four of these sera lost most, if not all of their neutralizing capacity, when heated to 56°C. for one-half hour.

However, the sera from one laboratory worker retained its neutralizing activity after it was heated at 56°C. for one-half hour. (Table II) The combined action of the heat labile and heat stable components

TABLE II

*Presence of heat resistant neutralizing substances in serum from laboratory worker*

| DILUTION | EXPERIMENT #1 | | EXPERIMENT #2 | | | | CONTROL NORMAL HEATED CHICKEN SERUM |
|---|---|---|---|---|---|---|---|
| | M.F.* Unheated | 1-18-49 Heated | M.F. Unheated | X-28-49 Heated | | | |
| | | | | 5 | 15 | 45 | |
| | | | | min. | | | |
| $10^{-0}$ | | | | | | | 0/4 |
| $10^{-6}$ | 0/4 | 0/4 | | | | | 0/5 |
| $10^{-7}$ | 0/4 | 0/4 | | | | | 4/4 |
| $10^{-8}$ | 0/4 | 0/4 | 0/4 | 0/3 | 0/5 | 1/4 | |
| $10^{-9}$ | 0/4 | 3/4 | 0/4 | 1/3 | 0/5 | 1/4 | |
| $10^{-4}$ | 0/4 | 4/4 | 0/4 | 1/4 | 1/3 | 3/4 | |
| $10^{-3}$ | 0/4 | 4/4 | 0/5 | 5/5 | 4/4 | 4/4 | |
| $10^{-2}$ | 0/4 | 3/4 | | | | | |
| $10^{-1}$ | | | | | | | |
| LD 50 of combination..... | <1.5 | 5.7 | <2.5 | 4.5 | 3.7 | 4.6 | 7.5 |
| LD 50 neutralized... | >6.0 | 1.8 | >5.0 | 3.0 | 3.8 | 2.9 | — |

* Virus used in this experiment was the CG 179 strain of Newcastle (13).

in this serum might be compared to the combined effect of complement and antibody. To test this possibility, we decomplemented freshly drawn sera by combining antigen with antibody in the presence of the fresh serum (15). Complement activity was found to be lost, when measured in a hemolytic system. However, there was no apparent loss of the neutralization power of two different fresh sera (Table III). This indicates that "complement" does not take part in the neutralization system, either in a person in whom both heat-resistant and heat-labile substances are demonstrable, or in one in whom neutralization cannot be demonstrated after the sera is heated. Furthermore, in tests using plasma instead of serum, the neutraliza-

TABLE III

*The effect of the removal of complement upon the neutralizing ability of human serum*

| DILUTION | EXPERIMENT #1, SERUM M.F. | | | HUMAN CONTROL HEATED | EXPERIMENT #2, SERUM F.I. | | | NORMAL CHICKEN CONTROL HEATED |
|---|---|---|---|---|---|---|---|---|
| | Unheated | Decomplemented | Heated | | Unheated | Decomplemented | Heated | |
| $10^{-9}$ | | | | 0/5 | | | | |
| $10^{-8}$ | | | | 0/5 | | | 2/5 | 2/6 |
| $10^{-7}$ | | 0/2 | 0/5 | 4/5 | | | 5/5 | 4/5 |
| $10^{-6}$ | | 0/5 | 1/5 | 5/5 | 0/5 | 0/5 | 5/5 | 5/5 |
| $10^{-5}$ | | 1/5 | 1/5 | | 1/5 | 2/5 | 5/5 | |
| $10^{-4}$ | 0/5 | 0/5 | 3/5 | | 3/5 | 4/5 | | |
| $10^{-3}$ | 1/5 | 1/5 | 5/5 | | 5/5 | 5/5 | | |
| $10^{-2}$ | 1/5 | 1/5 | | | | | | |
| $10^{-1}$ | 2/5 | 3/4 | | | | | | |
| LD 50 of combination. | 1.3 | 1.7 | 4.5 | 7.3 | 4.3 | 4.6 | 7.8 | 7.6 |
| LD 50 neutralized.... | 6.0 | 5.6 | 2.8 | | 3.3 | 3.0 | | |

TABLE IV

*A comparison of undiluted plasma and serum in the neutralization of Newcastle virus*

This individual in other tests demonstrated not to have heat stable neutralizing substance in serum

| DILUTION | F.I. FRESH SERUM | PLASMA PREPARED BY | | | CONTROL HEATED CHICKEN SERUM |
|---|---|---|---|---|---|
| | | Sodium Oxalate | Heparin | Silicone | |
| $10^{-9}$ | | | | | 0/4 |
| $10^{-8}$ | | | | | 1/4 |
| $10^{-7}$ | | 0/4 | 1/4 | 0/4 | 4/4 |
| $10^{-6}$ | 0/4 | 0/4 | 1/3 | 1/4 | |
| $10^{-5}$ | 0/4 | 1/4 | 1/4 | 0/4 | |
| $10^{-4}$ | 1/4 | 0/4 | 0/3 | 2/4 | |
| $10^{-3}$ | 1/3 | | | | |
| $10^{-2}$ | | | | | |
| LD 50 of combination*..... | 3.0 | <3.5 | 4.0 | 4.0 | 7.7 |
| LD 50 neutralized..... | 4.7 | >4.2 | 3.7 | 3.7 | — |

* Values calculated here are not completely accurate because of variable deaths, but are calculated to show high degree of activity of substances in serum and plasma.

tion titers showed no difference between fresh sera and plasma obtained by treating the blood with sodium oxalate, or with heparin, or by centrifugation in silicone-treated tubes.

There has been much discussion of the procedure of performing a neutralization test. Each test may have its particular requirements in regard to the dilution of the serum and the length of time that virus and serum have been combined before they are inoculated or injected in the test animal. The degree of specificity of the resulting

TABLE V

*The effect of dilution on heated human and heated immune chicken serum*

| DILUTION | HEATED HUMAN SERUM | | | | NORMAL HEATED CHICKEN SERUM | HEATED IMMUNE CHICKEN SERUM | | | | NORMAL HEATED CHICKEN SERUM |
|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{0}$ | $10^{-0.5}$ | $10^{-1}$ | $10^{-1.5}$ | | $10^{0}$ | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | |
| $10^{-9}$ | | | 0/5 | | 0/5 | | | | | 0/5 |
| $10^{-8}$ | 0/5 | 0/5 | 0/5 | 0/5 | 2/5 | | | | 0/4 | 2/5 |
| $10^{-7}$ | 0/5 | 1/5 | 2/5 | 2/5 | 4/5 | | | 0/3 | 3/4 | 4/4 |
| $10^{-6}$ | 1/5 | 1/5 | 2/5 | 5/5 | | | 0/4 | 1/4 | 4/4 | |
| $10^{-5}$ | 1/5 | 4/5 | | | | | 1/4 | 4/4 | 4/4 | |
| $10^{-4}$ | 3/5 | 5/5 | | | | 0/4 | 0/4 | 4/4 | | |
| $10^{-3}$ | | | | | | 3/4 | 4/4 | | | |
| $10^{-2}$ | | | | | | 1/4 | | | | |
| $10^{-1}$ | | | | | | 4/4 | | | | |
| LD 50 of combination.... | 4.5 | 5.5 | 6.2 | 6.8 | 7.8 | 2.5 | 3.6 | 5.7 | 7.2 | 7.8 |
| LD 50 neutralized.... | 3.3 | 2.3 | 1.6 | 1.0 | — | 5.3 | 4.2 | 2.1 | 0.6 | — |

effects may be modified by the use of different sera and different test animals. We here present an analysis of some of the factors which influence neutralization tests done with heated human and heated rabbit immune sera. These results are compared with similar experiments on heated immune chicken sera.

Undiluted human sera are used routinely in neutralization tests against the virus of yellow fever (19) with no evidence of "non-specific" results. It is known that such neutralizing substances are present in undiluted animal sera (7). In some situations, however, it may be necessary to use undiluted sera in order to detect antibodies. This makes it imperative to know the amount of virus neutralized by vari-

ous dilutions of sera in order to apply the neutralization test intelligently. In Table V and Fig. 2, we have compared the neutralizing effect of different dilutions of the heated human sera containing heat stable neutralizing substances, with a similar study on heated immune chicken sera. It may be seen that in both systems the slope of the

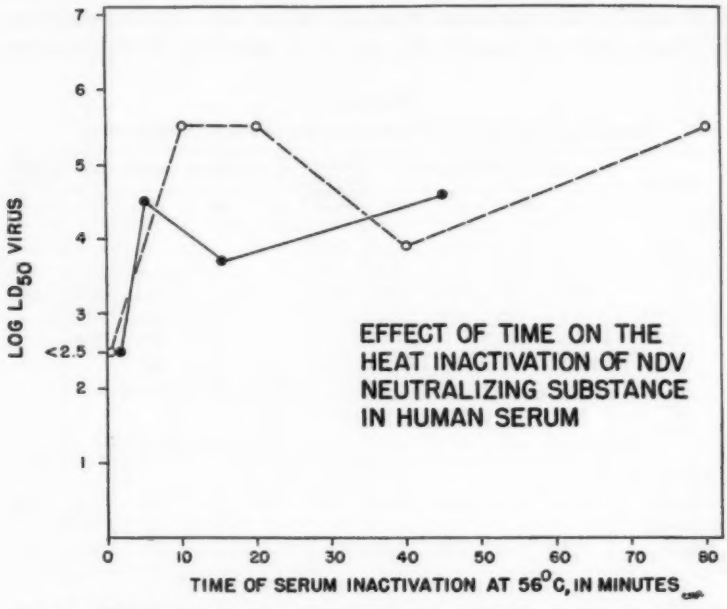


FIG. 1. The effect of heating on the neutralizing capacity of serum from M. F. Control mixtures of virus and heated normal chicken sera titer to $10^{-7.5}$ or better.

curve relating the neutralizing activity of the serum to the dilution of the serum is slightly greater than one. This means that a dilution of the serum used is accompanied by a slightly greater loss of neutralizing activity.

The effect of the time of combination of serum and virus upon the eventual neutralization titer has been studied and debated by a number of observers. Recent careful experiments on one system indicates that there is no effect greater than that produced by merely incu-

bating the virus for different time periods in normal sera (20). In our studies it seems apparent that an increased titer of neutralization is obtained by incubating heated "immune" human sera with virus at 35°C. before inoculation on embryos (Table VI). A time effect is also apparent with heated immune rabbit sera (Table VII).* In con-

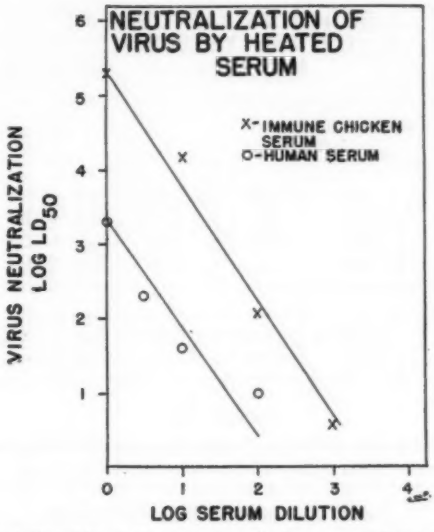


FIG. 2. The relationship of the amount of virus neutralized to the dilution of serum used in the test.

trast the effect of time of incubation of the mixture was not apparent when using immune chicken sera (Table VIII). The final results of a comparative experiment are presented in Table IX.

## ROUTE OF INOCULATION

A number of workers have inoculated mixtures of virus and sera into the allantoic sac and then by testing for the presence of hemagglutinins in the allantoic fluid two days later, determined the neutralization point of the virus. We have felt that this is not a safe

* No decrease in titer of virus was apparent when the dilutions of virus were incubated with normal heated rabbit sera.

procedure with Newcastle virus, for in our hands the virulent strain (CG 179) will not agglutinate red cells when it is grown in certain

TABLE VI

*The effect of time of combination of serum with virus upon the neutralization titer of heated human serum (F) and heated normal chicken serum*

| DILUTION OF VIRUS USED | SERUM PLACED ON EMBRYO BEFORE VIRUS | HEATED HUMAN SERUM (F) | | | | HEATED NORMAL CHICKEN SERUM | |
|---|---|---|---|---|---|---|---|
| | | Immediate inoculation | ½ hr, 4°C. | 1 hr, 35°C. | 2 hrs., 35°C. | Immediate inoculation | 2 hrs., 35°C. |
| $10^{-9}$ | | | | | | 0/5 | 1/5 |
| $10^{-8}$ | | | | | | 3/5 | 4/5 |
| $10^{-7}$ | | | | | | 5/5 | 4/4 |
| $10^{-6}$ | 1/1 | 4/5 | 2/2 | 1/4 | 0/5 | | |
| $10^{-5}$ | 2/2 | 5/5 | 3/3 | 2/3 | 2/5 | | |
| $10^{-4}$ | 5/5 | 3/3 | 3/3 | 4/5 | 2/4 | | |
| $10^{-3}$ | 5/5 | | 2/2 | 3/3 | 4/4 | | |
| LD 50 of combination | 6.5+ | 6.4+ | 6.5+ | 5.2 | 4.5 | 8.2 | 8.6 |
| LD 50 neutralized | <1.7 | <1.6 | <1.7 | 3.0 | 3.7 | | |

TABLE VII

*The effect of time of combination of serum with virus upon the neutralization titer of heated immune rabbit serum*

| | | RABBIT SERUM | | | | NORMAL CHICKEN SERUM | |
|---|---|---|---|---|---|---|---|
| | | Inoculation after incubation of 37°C. for: | | | | | |
| | | Immediate | ½ hr. | 1 hr. | 2 hrs. | 1 hr. | 2 hrs. |
| Exp. #1 | LD 50 of Combination | 5.0 | 5.0 | 4.5 | 3.2 | 8.0 | 7.5 |
| | LD 50 Neutralized | 3.0 | 3.0 | 3.5 | 4.8 | | |
| Exp. #2 | LD 50 of Combination | 5.3 | 4.5 | 4.0 | | 8.0 | |
| | LD 50 Neutralized | 2.7 | 3.5 | 4.0 | | | |

embryos. We have, however, seen no difference between a neutralization test when inoculated in the allantoic sac and followed to death of the embryo in the ensuing six days, as compared to inoculation on the membrane. Although previous studies have indicated that embryos incubated more than eleven days are slightly more resistant to

TABLE VIII

*The effect of time of combination of serum with virus upon the neutralization titer of immune chicken sera*

| DILUTION | EXPERIMENT #1 | | EXPERIMENT #2 | | CONTROL HEATED NORMAL SERA | EXPERIMENT #3 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ½ dilution unheated | | Heated undiluted | | | | | | |
| | Serum inoc. first-virus 5 min. later | ½ hr. 4°C. combination | Serum inoc. first-virus 5 min. later | ½ hr. at 4°C. before inoc. | | Immediate inoculation | 1 hr. 4°C. | 1 hr. 25°C. | 1 hr. 35°C. |
| $10^{-9}$ | | | | | 0/5 | | | | |
| $10^{-8}$ | | | | | 1/4 | | | | |
| $10^{-7}$ | | | | | 3/5 | | | | |
| $10^{-6}$ | | | | | 5/5 | | | | |
| $10^{-5}$ | 0/5 | 0/3 | 0/5 | 0/5 | | | | | |
| $10^{-4}$ | 3/4 | 0/3 | 1/4 | 1/5 | | 0/4 | 0/4 | 1/5 | 0/4 |
| $10^{-3}$ | 4/4 | 3/5 | 2/4 | 3/5 | | 3/5 | 3/4 | 3/4 | 2/5 |
| $10^{-2}$ | 5/5 | 2/2 | 5/5 | 4/5 | | 5/5 | 3/4 | 5/5 | 5/5 |
| $10^{-1}$ | | | | | | 4/4 | 4/5 | 5/5 | 5/5 |
| LD 50 of combination | 4.3 | 3.2 | 3.3 | 3.2 | 7.3 | 3.2 | 3.0 | 3.4 | 2.8 |
| LD 50 neutralized.... | 3.0 | 4.1 | 4.0 | 4.1 | | 4.1 | 4.3 | 3.9 | 4.5 |

TABLE IX

*The effect of time of combination of serum with virus upon the neutralization titer of several different heated sera tested simultaneously*

| SERUM | | COMBINATION INOCULATED | | |
|---|---|---|---|---|
| | | Immediately | After 1 hr. at 35°C. | After 2 hr. at 35°C. |
| Normal chicken (control) | LD 50 of combination | 8.2 | | 7.7 |
| Rabbit immune | LD 50 of combination | 4.4 | 3.7 | 2.5 |
| | LD 50 neutralized | 3.8 | 4.5 | 5.7 |
| Human immune | LD 50 of combination | 6.5 | 3.6 | 3.6 |
| | LD 50 neutralized | 1.7 | 4.6 | 4.6 |
| Chicken immune | LD 50 of combination | 4.3 | 3.3 | 3.8 |
| | LD 50 neutralized | 3.9 | 4.9 | 4.4 |

the virus (21), the prevalence of Newcastle Disease and the fact that antibodies go through the yolk and into the older embryo (22) make

the use of eleven day embryos theoretically better, and all tests here reported have been limited to ten or eleven day old embryos.

## PERFORMANCE OF NEUTRALIZATION TEST

From such experimental data we have concluded that a satisfactory neutralization test to determine the presence of antibodies to the virus of Newcastle Disease in human sera may be done if sera are heated to 56°C. for one-half hour, if undiluted sera are used against varying dilutions of virus, and if the mixture of virus and sera is incubated for one hour before inoculation of the embryo. Having determined this, we again tested for antibodies the sera of individuals who had had conjunctivitis due to Newcastle virus. From our file of sera we tested two from which virus had been recovered on two separate occasions each.[4] To these were added the sera from a recent case of unilateral conjunctivitis occurring in an individual working with infected birds.[5] All failed to show neutralization. Of the seven people who have worked in our laboratory and have had various degrees of contact with this virus, only one has neutralizing substances in the serum after heating. This has been true on repeated tests over a two year period. No serum was obtained from this individual before she had been working with the virus for some twelve months.

## DISCUSSION

It is now well established that a heat labile neutralizing substance is commonly present in human sera (8–11). The titer of this substance in our hands was not significantly lowered by decomplementing the sera, which confirms one report (8). On the other hand, loss of activity on decomplementing, as reported by another worker (10), might be explained by the lability of the substance itself. We have concluded that there is no evidence in favor of adding complement or attempting to use fresh sera for neutralization test when determining antibody

[4] The sera tested from Case #1 were obtained 1, 14, and 22 days after the onset of conjunctivitis and from Case #2 on 5 and 140 days after the onset.

[5] Courtesy of Dr. R. E. Shope, Merck Institute, Rahway, New Jersey. This serum was taken about one month after the onset of a characteristic conjunctivitis. Virus isolation was not attempted until late in the course of the infection, and was then unsuccessful.

rise. In order, however, to further check this particular point, we inoculated two monkeys intranasally with undiluted virus and determined their neutralization titer on fresh and heated sera, before and after (16 and 20 days) inoculation. One monkey developed a brief temperature rise ten days after inoculation. The acute and convalescent sera were run simultaneously, the acute serum having been frozen immediately after separation. A similar study was carried out on two rabbits with sera taken before injecting virus and fourteen days afterwards. No consistent evidence was obtained indicating a potentiating effect of the heat labile factor (present in these sera also) on the convalescent sera above and beyond that expected from adding the neutralization produced by the antibodies to that produced by the heat labile factor. Actually the monkeys failed to develop any detectable heat stable neutralizing substances, while the rabbits did so following their intravenous inoculation of virus.

## SUMMARY

The widespread prevalence of Newcastle Disease virus among chickens and the occasional infection of man by this virus makes it important that adequate tests for the presence of neutralizing antibodies in human sera be evolved. The uniform presence of a heat labile neutralizing substance in all the human sera tested is established. This substance is not removed by decomplementing, and it remains effective in plasma obtained by treating blood with sodium oxalate, with heparin, or by centrifugation in silicone coated tubes. This indicates that all human sera should be heated to 56°C. for one-half hour before using them in the neutralization test.

The factors involved in the neutralization of Newcastle Disease virus as tested by inoculation of chick embryos are analyzed. The slope of the curve relating the neutralizing activity of the serum to the dilution of the serum is slightly greater than one. This means that a dilution of the serum used is accompanied by a slightly greater loss of neutralizing activity. This was true for both immune chicken serum and one "immune" heat-activated human serum. Incubation of either this heat-inactivated human serum or a similarly treated immune rabbit serum with the virus before inoculation of the embryo

with the mixture increased the neutralization titer. No similar effect was apparent when chicken immune sera were used.

Of seven sera from personnel in close contact with the virus of Newcastle Disease, only one had heat stable neutralizing substances. These have been consistently demonstrable in this person for two years. None of three cases of conjunctivitis due to Newcastle virus developed antibodies measurable by this improved test.

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# DEPRESSION OF SYNAPTIC TRANSMISSION THROUGH SYMPATHETIC GANGLIA FOLLOWING TEMPORARY OCCLUSION OF THE AORTA: AN EFFECT OF ENDOGENOUS ADRENALIN[1]

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During an investigation of the effects of lack of circulation on synaptic mechanisms in sympathetic ganglia, ischemia was produced by clamping the ascending aorta. It was thus found that there was usually no depression of synaptic transmission during circulatory arrest for 1 to 10 minutes. However, when the circulation was restored by releasing the aorta after ischemia of those durations, there ensued a period during which many ganglion cells failed to discharge impulses in response to preganglionic nerve impulses. This is strikingly illustrated by the experiments shown in figures 1 and 2.

It is the purpose of this paper to describe and analyze this depression of response of sympathetic ganglion cells, which appears after circulation is restored at the end of relatively short periods of aortic occlusion. This depression, which occurs *after* the ischemia is terminated, must be distinguished from depression which occurs *during* ischemia of longer duration. In the experiments to be described in this paper the circulation was not arrested for a time sufficient for transmission to be depressed during the ischemia. Previous observations have shown that such ischemic depression usually does not begin until circulation has been stopped more than 10 minutes (Bronk, Larrabee, and Gaylor, 1948). During the early minutes of circulatory arrest there is, in fact, an increase, rather than a decrease, in the number of ganglion cells responding to preganglionic nerve impulses. (Posternak, Lar-

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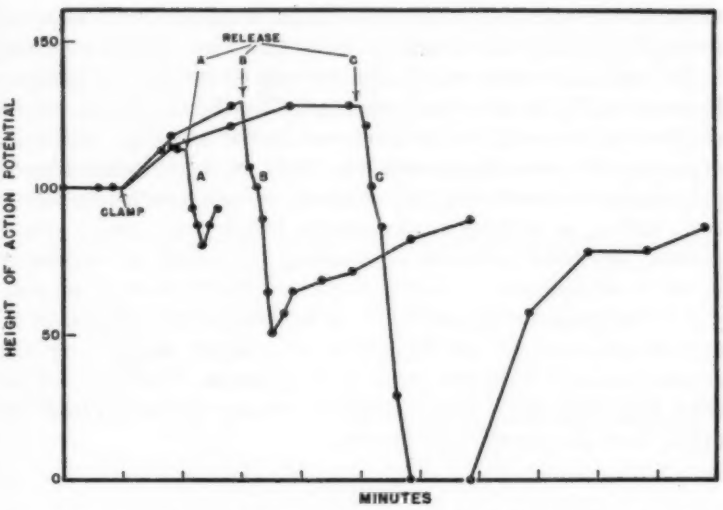


FIG. 1. Increase in number of sympathetic ganglion cells responding to synaptic excitation during occlusion of the ascending aorta and decrease in number responding following release of the aorta. The aorta was clamped at first arrow in each of three successive trials and released after various times as indicated by later arrows. Number of cells responding was measured by height of action potential in postganglionic nerve from stellate ganglion in response to an occasional volley of preganglionic nerve impulses.

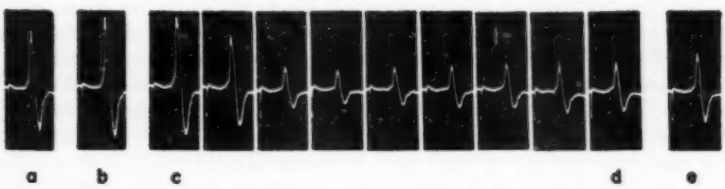


FIG. 2. Records from an experiment similar to that of Figure 1, showing action potentials in the postganglionic nerve of the stellate sympathetic ganglion, each elicited by a single stimulus to preganglionic fibers in the thoracic sympathetic trunk. The ascending aorta was clamped following record *a* and released three minutes later, following record *b*. Records *b* to *d* at 10 second intervals: 90 seconds between *d* and *e*.

rabee, and Bronk, 1947). This is indicated in figures 1 and 2, for example, by an increase in height of the postganglionic action potential.

The conditions under which the initiation of impulses at synapses by presynaptic impulses was suppressed after the circulation was restored were not conditions which occur in the normally functioning organism. Nor were they conditions likely to be reproduced under pathological circumstances. The observations here reported are significant, rather, as defining a phenomenon which occurs under experimental conditions produced in studying the effects of circulatory arrest on mechanisms of synaptic excitation. Furthermore, as an analysis of the phenomenon will show, these observations emphasize by experimental example the dependence of synaptic processes on substances produced in remote parts of the organism. Possibly a similar effect may occur under more natural conditions; this cannot be determined from the present experiments.

## METHODS

Experiments were performed on sympathetic ganglia of cats anesthetized with sodium pentobarbital (Nembutal). Most observations were made on the stellate ganglion. Its preganglionic nerve (the thoracic sympathetic trunk) and one of its postganglionic nerves (the inferior cardiac nerve) were prepared for electrical stimulation and electrical recording by methods previously described (Bronk, Larrabee and Gaylor, 1948). Since this required extensive removal of the chest wall, the animals were maintained on artificial respiration.

Volleys of preganglionic nerve impulses were elicited by single supramaximal stimuli applied to the thoracic sympathetic trunk at intervals of 10 or more seconds. Under the conditions of these experiments the height of the action potential recorded in the inferior cardiac nerve is a measure of the number of ganglion cells responding to the synaptic excitation (Bronk, Larrabee, and Gaylor, 1948).

In some experiments the ganglion was perfused in a way previously described (Bronk, Tower, Solandt and Larrabee, 1938). The perfusion solution contained 0.1% glucose and 3% gelatin (prepared from Knox P20 solution) in addition to the usual constituents of Ringer's fluid. It was equilibrated with 95% $O_2$ and 5% $CO_2$, and was buffered with bicarbonate. To prevent blood from reaching the ganglion through collateral sources the animal was either bled to death, or the perfusion pressure was kept considerably higher than the arterial blood pressure.

## RESULTS

Figure 2a shows the action potential of impulses discharged over the postganglionic nerve in response to a volley of preganglionic impulses sent into a ganglion with normal circulation. When the flow of

blood was stopped by clamping the ascending aorta, there was the usual increase in number of ganglion cells responding to a volley of preganglionic impulses, indicated by the increased height of action potential in figure 2b. This increase gave way to a great reduction in number of cells responding after the aorta was released (figures 2c to d). The depression of response lasted for more than a minute.

A similar depression was observed in each of 19 stellate ganglia fol-

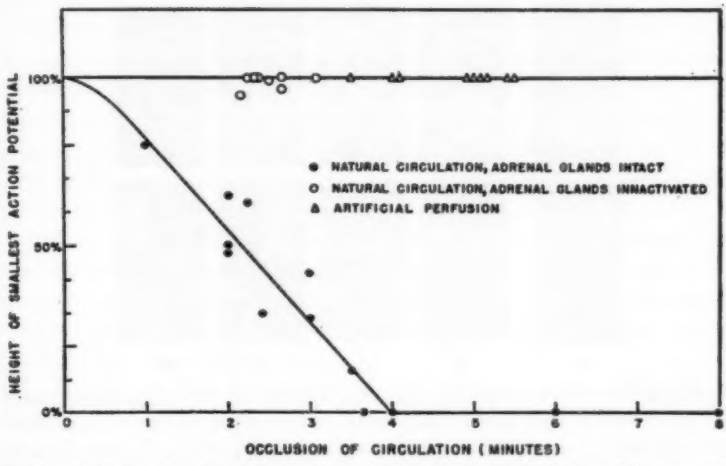


FIG. 3. Depression of synaptic transmission following occlusion of the ascending aorta for various periods, absence of such depression following aortic occlusion when the adrenal glands were inactivated, and similar absence of depression following temporary interruption of artificial perfusion. Ordinate represents height of postganglionic action potential in response to a volley of preganglionic impulses at the time of deepest depression after each occlusion, expressed as per cent of control height before occlusion.

lowing restoration of the circulation by releasing a clamp on the ascending aorta. It was also seen in experiments on the inferior mesenteric ganglion. The amount of depression increased progressively with the duration of circulatory arrest (fig. 1 and lower curve of fig. 3), until, after 4 or more minutes of aortic occlusion, there was always a period during which none of the cells responded to volleys of preganglionic impulses.

The number of ganglion cells responding was depressed promptly

after release of the aorta in all cases where the heart was beating effectively. In several other experiments the ventricles at first failed to contract, but finally resumed their normal action spontaneously or following massage of the heart; in one instance this was as long as 4 minutes after removal of the aortic clamp. In these cases synaptic transmission was unaffected by release of the aorta, but it was depressed upon subsequent restoration of the heart beat.

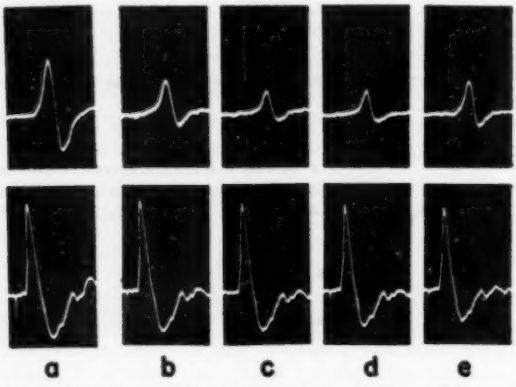


FIG. 4. Depression of synaptic pathways through the stellate ganglion (shown by upper records, from postsynaptic axons in inferior cardiac nerve), compared with unimpaired conduction over axons traversing ganglion without synapse (shown by lower records, from cervical sympathetic trunk). Stimuli were applied to the thoracic sympathetic trunk for both sets of records. Ascending aorta had been clamped some time before the first records, and was released after 2 min. 20 sec., following record *a*. Records *a* to *d* at 10 sec. intervals; 75 sec. between *d* and e. Each lower record was taken immediately after the corresponding upper record.

There was no block of conduction along axons which traverse the ganglion without synapse, such as those which pass from the thoracic trunk into the cervical sympathetic nerve (fig. 4). Accordingly it may be concluded that depression of transmission over the synaptic pathway was due to an effect on the synaptic regions within the ganglion, rather than to an interference with conduction of impulses over the axons entering or leaving the ganglion. It is, of course, possible that there was a general reduction of irritability of all intraganglionic nerve

tissue, axons as well as synaptic structures. If so, the amount of depression was sufficient to block trans-synaptic excitation but not axonal conduction.

*Analysis of mechanisms*

A number of possible mechanisms for depression of synaptic processes, caused by clamping and releasing the aorta, may now be considered.

1. The depression of response was not simply a phase through which the nerve cells passed during recovery from ischemia. This was shown by the fact that the depression observed in animals with natural circulation could not be reproduced in ganglia which were perfused. In no case where perfusion was stopped and then restored after periods as long as 5½ minutes, was there a detectable amount of depression (triangles in fig. 3).

2. The depression was not a direct effect on the ganglion caused by changes in blood pressure, which, for example, may have been temporarily elevated following release of the aorta. This was shown by absence of any comparable effect of similar changes in pressure during artificial perfusion experiments.

3. The depression did not result from changes in the discharge of impulses from the central nervous system into the ganglion over intact rami communicantes, for the effect was still obtained after special care was exercised to section all rami communicantes innervating the ganglion under observation.

4. Finally, a group of experiments suggested that the depression of synaptic transmission was caused by some substance released into the blood in other parts of the body during the period of ischemia and subsequently transported to the ganglion after release of the aorta. For example, when the ganglion was artificially perfused, so that no blood could enter it after release of the aorta, then no depression occurred.

We at first considered that the depression might be due to some change occurring in blood which was stagnant in the heart while the aorta was clamped. This was, however, shown not to be the case by withdrawing 2 cc of blood from the ventricles during the period of occlusion and injecting this blood into the cannula supplying fluid to

a perfused ganglion. In each of several experiments such blood had no depressant effect on synaptic transmission.

In other observations blood was withdrawn from the heart while it was beating *after* release of the aorta, at a time when synaptic transmission was depressed. When this blood was injected into the perfusion system supplying a ganglion it caused a large reduction in the postsynaptic response in one of two experiments, (fig. 5) and a smaller, but unequivocal depression in the other. This was evidence that a depressant substance was transported to the ganglion from some other part of the body after the circulation was restored.

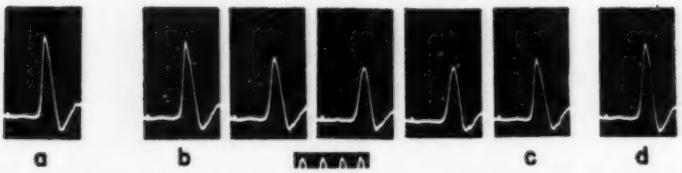


FIG. 5. Action potentials in postganglionic nerve of a perfused stellate ganglion following stimulation of preganglionic fibers. The number of ganglion cells responding was reduced by injecting 2 cc. of blood into the perfusion cannula after record *a*. This blood had been withdrawn from the left ventricle shortly after releasing the ascending aorta, which had been clamped for three minutes. Records *a* to *c* at 5 sec. intervals; 30 sec. between *c* and *d*. Time scale for individual records shown in $\frac{1}{60}$ sec. intervals.

Evidence was obtained that the depressant substance entered the blood in some region more caudal than the heart. In one experiment the inferior vena cava as well as the ascending aorta was occluded. When, after more than two minutes of occlusion, the aorta alone was released, there was no depression of synaptic transmission. Depression followed subsequent release of the vena cava, however (fig. 6). In other experiments it was found unnecessary to render the upper portion of the body ischemic in order to produce the effect. Thus depression followed release after a period of simultaneous occlusion of the descending aorta and inferior vena cava. (Clamping the descending aorta alone, while leaving open the inferior cava, did not cause sufficient ischemia to be followed by ganglionic depression.)

The depressant substance arose elsewhere than in the gastrointes-

tinal tract, spleen, or pancreas, for depression was still caused by clamping and releasing the ascending aorta after removal of these organs. Moreover the substance did not appear to come from the hind limbs, for no depression followed release of blood which had been held

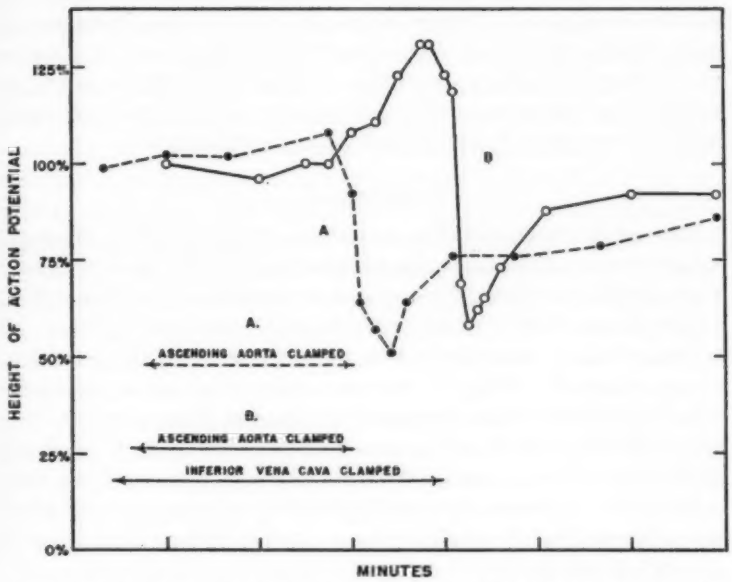


FIG. 6. Delay in depression of synaptic transmission after release of ascending aorta, caused by occlusion of inferior vena cava. Ordinates are heights of postganglionic nerve action potentials in response to occasional stimuli to the preganglionic nerve. Two successive trials in the same experiment. In A the ascending aorta alone was clamped, and depression promptly followed its release. In B both the ascending aorta and the inferior vena cava were clamped. There was no depression following release of the aorta, until the vena cava was also released, thus allowing blood to return from the lower portions of the body.

there in prolonged stasis by tourniquets with or without mechanical trauma to the limbs.

Finally, direct evidence was obtained that the depressant substance came from the adrenal glands. When these glands were isolated by ligatures, there was never any depression of synaptic transmission fol-

lowing release of the aorta (unfilled circles in fig. 3). In our first attempts to perform such an experiment the blood pressure dropped to a very low level after the ligatures were placed to isolate the adrenals, so that conditions were not reasonably comparable to animals with adrenal glands intact. However, in two other animals, in which the glands were approached retroperitoneally, the blood pressure remained at a satisfactory level (greater than 100 mm. Hg in one, and 75 mm. in the other), but still no depression could be produced. Therefore, we consider that dependence of the depression of synaptic transmission on the adrenal glands has been adequately demonstrated.

## DISCUSSION

The substance liberated from the adrenal glands which depressed synaptic transmission was presumably adrenalin.[3] Marrazzi has shown that adrenalin depresses the response of sympathetic ganglion cells to preganglionic nerve impulses, both following intravenous injection, and following release from the glands by stimulation of the splanchnic nerves (Marrazzi, 1939a, b). We have similarly caused depression by injecting as little as 2.5 micrograms of adrenalin intravenously and by perfusing the ganglion with concentrations as low as $1:10^7$. Bulbring (1944) has observed depression with even lower concentrations. Marrazzi (1947) has shown that only synaptic pathways are blocked by adrenalin, and not axons traversing a ganglion without synapse, as in the present experiments.

Our findings could be explained by assuming that adrenalin was secreted no more rapidly during the period of ischemia than before, but merely accumulated in high concentration in blood which was stagnant in the adrenal glands. There are, however, several mechanisms which we would expect to cause an increase in rate of adrenalin secretion under the conditions of our experiments, especially since such an increase has been observed during asphyxia and hypoxia (Cannon and Hoskins, 1911; Kellaway, 1919; Houssay and Molinelli, 1926). One

[3] There is evidence that all natural "adrenalin" is actually a mixture of noradrenalin and true adrenalin. (e.g. Goldenberg et al, 1949; Tullar, 1949; Auerbach and Angell, 1949). No distinction has been made between these two components in this investigation, so that the term "adrenalin" is used to include possible mixtures.

possible mechanism is the direct stimulating effect of anoxia caused by interference with the oxygen supply to the adrenal glands (Bulbring, Burn, and De Elio, 1948). Another is the effect of increased discharge of impulses over the splanchnic nerves, which has been observed by electrical recording when the ascending aorta is clamped (Posternak, 1948).

The reduction of arterial blood pressure caused by clamping the aorta presumably contributed to this increase in activity of the sympathetic nervous system. Thus a drop in blood pressure could result in accelerated adrenalin secretion due to reflexes initiated in cardiovascular pressure receptors. Such reflex control, however, did not appear to be an important factor in our experiments, since usually the depression of synaptic transmission was not significantly reduced after the reflex centers in the medulla oblongata had been inactivated by prolonged deprivation of their blood supply. This was achieved by ligating the vertebral and carotid arteries; in some cases the vagus nerves were also cut to further assure elimination of cardiovascular reflexes.

A more important mechanism for nervous liberation of adrenalin in our experiments would, therefore, appear to be the increase in sympathetic activity which Alexander (1945) has found to result from asphyxia of the spinal cord. It is pertinent to note that complete asphyxia of the cord in the regions from which the splanchnic nerves arise cannot be caused by clamping either the descending thoracic aorta or the abdominal aorta (Tureen, 1936). These findings are therefore in agreement with our observations that temporary occlusion of either of these vessels fails to initiate the profound depression of synaptic transmission which we have observed after the total ischemia produced by occlusion of the ascending aorta.

## SUMMARY

Synaptic transmission through a sympathetic ganglion became depressed when the ascending aorta was released following occlusion for 1 or more minutes. This was shown by recording the action potentials of impulses discharged from the ganglion cells in response to volleys of presynaptic impulses, initiated by electrical stimulation of the preganglionic nerve.

The degree of depression occurring on release of the aorta increased with the duration of occlusion, synaptic transmission being completely blocked for many seconds if the occlusion had lasted more than 4 minutes. There was no block of conduction of impulses over axons traversing the ganglion without synapse.

Various experiments showed that this depression of synaptic transmission was not simply a phase of recovery of the nerve cells after ischemia, nor was it caused by the changes in blood pressure, acting either directly or reflexly on the sympathetic ganglia.

The depression was due to a chemical change in the blood, whose origin was caudal to the heart, but not in the gastro-intestinal tract, spleen, pancreas, or hind limbs.

The substance depressing synaptic transmission was finally shown to arise in the adrenal glands, since the effect was lacking after removal of these organs. Presumably the substance was adrenalin (or nor-adrenalin), which others have shown to depress synaptic transmission in sympathetic ganglia. Adrenalin (and nor-adrenalin) was probably released under the conditions of these experiments by a discharge of impulses over the splanchnic nerves initiated by ischemia of the spinal cord.

Thus our observations can be explained by the liberation of adrenalin (or nor-adrenalin) from the adrenal glands while the aorta was clamped, and its subsequent transport to the sympathetic ganglia after the circulation was restored.

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# THE APPEARANCE OF VITAMIN $B_{12}$ ACTIVITY IN URINE AFTER ORAL AND INTRAMUSCULAR ADMINISTRATION TO MAN*

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Vitamin $B_{12}$ appears to be an essential nutritional factor, administration of which may overcome the manifestations of pernicious anemia (1). Evidence available at the present time suggests that in patients with this disease there is impaired absorption of vitamin $B_{12}$ from the gastrointestinal tract. Oral administration of the vitamin, in amounts equivalent to several times the minimum effective parenteral dose, is without therapeutic effect in patients with pernicious anemia. However, when vitamin $B_{12}$ is given orally with normal gastric juice, a therapeutic response may be obtained with amounts of the vitamin which are ineffective when given alone (2, 3). The manner in which gastric juice brings about this effect remains to be elucidated. It has been reported that normal gastric juice contains a non-dialyzable, heat labile substance which combines with vitamin $B_{12}$ to form a complex from which $B_{12}$ cannot be dissociated by dialysis (4). However, other studies on mixtures of gastric juice and vitamin $B_{12}$ have failed to provide any evidence that normal gastric juice produces a detectable alteration in vitamin $B_{12}$ or in its microbiological activity (5). Presumably, gastric juice exerts its effect by enhancing the absorption of vitamin $B_{12}$ from the intestinal tract.

In previously reported studies, hematologic response in untreated patients with pernicious anemia has been the only measure of the absorption of vitamin $B_{12}$ employed. We have attempted to use the $B_{12}$ activity of the urine, estimated by microbiological assay, as a measure of the absorption of the vitamin. The experiments to be described show that following the parenteral administration of vitamin

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$B_{12}$ to normal subjects and to patients with pernicious anemia, there was a prompt and enormous increase in the $B_{12}$ activity of the urine. This observation suggests that vitamin $B_{12}$ is excreted by the kidneys. It was thought, therefore, that the $B_{12}$ activity of the urine would provide an index of the degree of absorption of $B_{12}$ from the gastrointestinal tract following its oral administration. However, the experimental data show that the urine of normal individuals as well as that of patients with pernicious anemia displayed no appreciable $B_{12}$ activity after the ingestion of relatively large amounts of the crystalline vitamin.

## METHODS

The experimental studies were carried out on ambulatory normal young adult male subjects and on hospitalized patients with typical pernicious anemia in relapse. In most of the experiments, a meat-free diet relatively deficient in vitamin $B_{12}$ was provided, but daily intake of one egg and 3 glasses of milk was permitted. All of the urine produced by each subject was collected under toluol as preservative. A solution of crystalline vitamin $B_{12}$ was administered either orally or intramuscularly. Specimens of urine were assayed for vitamin $B_{12}$ activity by a microbiological method[10] employing *L. Leichmanii* as the test organism (6). In order to test out ability to determine vitamin $B_{12}$ in normal urine with this microbiological method, known amounts of this vitamin were incorporated in the urine, within the range of that present in the urine specimens. Our results demonstrate that the recovery was within 20% of the calculated values.

In two of the experiments biotin was incorporated with the vitamin $B_{12}$ solution and given to the subjects either orally or parenterally, because this vitamin is known to appear in both urine and stool specimens. Incorporation of biotin, therefore, serves as a guide to the adequacy of collection. Specimens of urine were collected and assayed for both vitamins. Biotin was determined with a method employing *Lactobacillus arabinosus* (7).

## RESULTS

1. *The vitamin $B_{12}$ activity of the urine of normal subjects following intramuscular injection of the vitamin.* Two normal subjects were maintained on a normal diet and total urine collections made. After a 24 hour control period, a solution of 100 micrograms of crystalline $B_{12}$ was injected intramuscularly into the deltoid. Urine specimens collected for one day prior to and for 2 days following the injection were assayed for vitamin $B_{12}$ activity. The results of these assays are shown in table 1. There was no appreciable $B_{12}$ activity detectable in the

urine prior to the parenteral administration of the vitamin. However, in the first 12 hours following the injection, the $B_{12}$ activity of the urine was equivalent to the activity of about 50% of the injected material.

TABLE 1

*The appearance of vitamin $B_{12}$ activity in the urine of normal individuals after intramuscular injection*

| HOURS FROM ADMINISTRATION | SUBJECT A | | SUBJECT B | |
|---|---|---|---|---|
| | mγ/ml[1] | %[2] | mγ/ml[1] | %[2] |
| −24 | 3.0 | 0 | 0.1 | 0 |
| 0 | | | | |
| 1 | 289 | 8 | 63 | 7 |
| 2 | 368 | 18 | 77 | 20 |
| 4 | 288 | 37 | 72 | 38 |
| 8 | 114 | 49 | 13 | 44 |
| 12 | 2 | 50 | 4 | 46 |
| 24 | 0.3 | 50 | 0.2 | 46 |
| 48 | 0.06 | 50 | 0.08 | 46 |

TABLE 2

*The appearance of vitamin $B_{12}$ activity and of free biotin in the urine of a patient with untreated pernicious anemia after intramuscular injection*

| HOURS FROM ADMINISTRATION | % EXCRETION[3] | |
|---|---|---|
| | Biotin | $B_{12}$ |
| 0 | 0 | 0 |
| 4 | 24 | 37 |
| 8 | 39 | 63 |
| 18 | 58 | 66 |
| 27 | 82 | 68 |
| 75 | 96 | 72 |
| 90 | 96 | 72 |

2. *The vitamin $B_{12}$ activity of the urine of patients with untreated pernicious anemia in relapse following intramuscular injection of the vitamin.* Three patients with untreated pernicious anemia were chosen for study. Two of them received 100 micrograms of crystalline vitamin $B_{12}$ intramuscularly, and the third was given 120 micrograms of $B_{12}$ and 10 mg. of biotin. Our data demonstrated that most of the

vitamins administered by this route appeared in the urine. The excretion data of the third patient, typical of all the three, are given in table 2.

3. *Vitamin $B_{12}$ activity of the urine of normal subjects following oral administration of the vitamin.* This experiment was identical with that in section 1 except that two normal subjects were given 100 micrograms of crystalline vitamin $B_{12}$ by mouth instead of parenterally. In this instance, however, urine collected over a period of 48 hours after administration of $B_{12}$ showed no detectable $B_{12}$ activity.

TABLE 3

*The appearance of vitamin $B_{12}$ activity in urine of normal subjects after oral or parenteral administration*

| GROUP A | | | GROUP B | | |
|---|---|---|---|---|---|
| Route | Hours from administration | % excretion[4] | Route | Hours from administration | % excretion[4] |
| Intramuscular | −24 | 0 | Oral | −24 | 0 |
| | 0[5] | 0 | | 0[6] | 0 |
| | 2 | 48 | | 24 | 0 |
| | 4 | 73 | | 48 | 0 |
| | 8 | 94 | | 72 | 0 |
| | 24 | 139 | | 96 | 0 |
| | 96 | 139 | Intramuscular | 0[5] | 0 |
| Oral | 0[6] | 0 | | 2 | 46 |
| | 24 | 0 | | 4 | 66 |
| | 48 | 0 | | 8 | 78 |
| | 72 | 0 | | 24 | 78 |
| | | | | 96 | 78 |

4. *Comparison of the vitamin $B_{12}$ activity of the urine of normal subjects after oral and after parenteral administration of relatively large amounts of the vitamin.* Six normal subjects participated in this experiment. For 10 days meat, poultry, cheese, tomatoes and sea food were excluded from their diets, and soy bean cake was substituted as the main source of protein. However, each subject was allowed one egg and one quart of milk daily. After a 24 hour control period, 3 of the subjects were given by intramuscular injection a solution containing 500 micrograms of crystalline vitamin $B_{12}$ and an equal amount of

biotin, and the remaining 3 subjects were given the same amount of the vitamin mixture orally. Urine was collected and assayed for the activities of both vitamins. After 6 days the vitamin mixture was

TABLE 4

*Urinary excretion of free biotin after oral or parenteral administration*

| GROUP C | | | GROUP D | | |
|---|---|---|---|---|---|
| Route | Hours from administration | % excretion[7] | Route | Hours from administration | % excretion[7] |
| Intramuscular | −24 | 0 | Oral | −24 | 0 |
| | 0[8] | 0 | | 0[9] | 0 |
| | 2 | 24.2 | | 12 | 28 |
| | 4 | 32 | | 24 | 31 |
| | 8 | 43.5 | | 36 | 35.4 |
| | 12 | 47.2 | | 48 | 38 |
| | 24 | 53 | | 72 | 39 |
| | 48 | 62 | | | |
| | 96 | 62 | Intramuscular | 0[8] | 0 |
| | | | | 2 | 20.6 |
| Oral | 0[9] | 0 | | 4 | 24 |
| | 12 | 36 | | 8 | 31.1 |
| | 24 | 39 | | 12 | 34.8 |
| | 36 | 41.8 | | 24 | 36 |
| | 48 | 42 | | 48 | 42 |
| | 72 | 42 | | 96 | 42 |

[1] Millimicrograms of vitamin $B_{12}$ per ml. urine

[2] % of ingested vitamin $B_{12}$ activity which appeared in the urine

[3] % of ingested vitamin which appeared in the urine

[4] The average % of the ingested vitamin $B_{12}$ activity which appeared in the urine of three subjects

[5] Time of intramuscular injection

[6] Time of oral administration

[7] The average % of the ingested biotin excreted by three subjects

[8] Time of intramuscular injection

[9] Time of oral administration

[10] The authors are indebted to Dr. Asger Langlykke of E. R. Squibb and Sons for the microbiological assays for $B_{12}$.

again administered, 500 micrograms of each being given orally to those subjects who had at first received it parenterally. Similarly the subjects who had previously received the vitamin orally were now given

it by intramuscular injection. Urine was again assayed for these vitamins. The results of bio-assay for vitamin $B_{12}$ (see table 3) indicate that this vitamin did not appear in the urine of any of the normal subjects following oral administration of 500 micrograms of vitamin $B_{12}$. However, in every case, parenteral injection was followed by the prompt appearance of $B_{12}$ activity in the urine. The $B_{12}$ activity of the urine diminished rapidly about 8 hours after the injection and in 3 of the subjects became negligible within 24 hours.

At the conclusion of this experiment, each of the 6 subjects was given a high protein diet containing 400 grams of beef per day (one subject received pork and chicken instead of beef) for a period of 4 days. During this period, there was no appreciable $B_{12}$ activity in the urine.

As part of a collateral study, biotin was incorporated with the vitamin $B_{12}$ solution in equal amounts and the urine samples were assayed for biotin. The results (see table 4) demonstrate that unlike vitamin $B_{12}$, free biotin appeared in the urine of all individuals to whom it was administered either orally or parenterally. The recovery of free biotin in urine was not complete. Part of the ingested biotin probably appeared in the stool (not determined), and the remainder was conjugated (7) and therefore not available for microbiological assay without hydrolysis. The per cent of recovery of free biotin appeared to be greater after intramuscular injection than after oral administration.

## DISCUSSION

No appreciable vitamin $B_{12}$ activity, as measured by microbiological assay, could be demonstrated in the urine of normal subjects or of patients with pernicious anemia. A diet presumably high in vitamin $B_{12}$ did not increase the $B_{12}$ activity of the urine of normal individuals. Furthermore, oral administration of amounts of vitamin $B_{12}$ as large as 500 micrograms was not followed by the appearance of $B_{12}$ activity in the urine of normal subjects. On the other hand, intramuscular injection of vitamin $B_{12}$ was regularly followed by the rapid appearance of $B_{12}$ activity in the urine both in normal subjects and in patients with pernicious anemia. In the preliminary studies thus far carried out, no significant difference has been observed in the urinary response of normal subjects and of patients with pernicious anemia.

Such a difference in the effect of route of administration was not observed when biotin was administered to some of our subjects.

These observations suggest that rapid renal excretion of vitamin $B_{12}$ occurs when that substance enters the body fluids. Unfortunately, chemical methods for identification of $B_{12}$ are not available, and we are unable to prove that the $B_{12}$ activity of the urine of our subjects was brought about by the same substance which had been injected. Furthermore, we are likewise uncertain that the absence of microbiological activity in urine indicates the retention of this vitamin by man.

After demonstrating the striking increase in $B_{12}$ activity of the urine following the parenteral injection of the vitamin, we had anticipated that the same result would follow oral administration of vitamin $B_{12}$ to normal individuals. We expected that patients with pernicious anemia would not absorb the vitamin from the gastrointestinal tract, and that in these individuals there would be little or no excretion of $B_{12}$ in the urine after its oral administration. We were therefore surprised to observe the failure of appearance of $B_{12}$ activity in the urine of the normal subjects after large oral doses. This observation might indicate that vitamin $B_{12}$ is poorly absorbed even by normal individuals. Other alternative explanations can be suggested, however. For example, it is conceivable that vitamin $B_{12}$ is altered in some way during its passage through the gastrointestinal tract so that it is no longer capable of being excreted by the kidneys. Or $B_{12}$ might undergo some change during its absorption which would render it ineffective in meeting the nutritive requirements of the organism employed in the assay.

## SUMMARY

1. The urine of normal subjects and of untreated patients with pernicious anemia displayed no vitamin $B_{12}$ activity as measured by microbiological assay.

2. Oral administration of a diet rich in vitamin $B_{12}$ or of single oral doses of vitamin $B_{12}$ as large as 500 micrograms was not followed by the appearance of detectable vitamin $B_{12}$ activity in the urine of normal subjects.

3. Intramuscular injection of vitamin $B_{12}$ was regularly followed by a striking increase in the $B_{12}$ activity of the urine both in normal subjects and in patients with untreated pernicious anemia.

4. The administration of biotin by either the oral or the parenteral route was followed by the appearance of this vitamin.

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# BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

*Die Röntgenbildanalyse: Eine Röntgendiagnostische Anleitung für Studierende und Ärzte.* Von. Prof. Dr. ERICH SAUPE. 2. Auflage, Neu bearbeitet von W. E. Baensch, Washington, D. C. Pp. 244, with 216 illustrations, 39 marks. *Georg Thieme Verlag, Stuttgart.*

This book was written primarily as a guide to the fundamentals of film interpretation and is not to be considered as a textbook of diagnostic roentgenology. Consequently disease processes are presented from the point of view of the types of shadow or shadows they are likely to produce on film or fluoroscopic screen. Narrowness, which might otherwise be inherent in such a presentation, is partially averted by frequent practical explanations drawn from the evident wide experience of the author. One wonders why this somewhat practical approach has not been more frequently employed in English and American works in this field.

The book is divided into 2 parts. In section A the physical factors involved in the production of x-ray film and fluoroscopic images are considered. These factors are not subjected to a rigid analysis but are described in rather simple terms and by diagrams so that they should be readily grasped by the beginner. This is followed by a discussion of such interesting and related topics as: The meaning of functional x-ray diagnoses; the possibilities of making an etiological diagnosis from roentgen studies; information which should be drawn from serial x-ray films and the general relationship of fluoroscopy to films in roentgen studies.

Section B deals with the roentgenology of the various body systems. A moderately extensive correlation between the roentgen appearance of disease and the course of the disease is attempted. The subject matter in general reflects the opinions and practice of the continental school of radiologists. Some of the material included in the sections on the chest and digestive tract appears to have originated from Dr. Baensch's Clinic in Washington and as such represents the practice of roentgenology as we know it in this country. Opinions of a controversial nature are not included.

A valuable feature is the inclusion of many typical detailed reports of roentgen studies which would serve as models for the beginner who is faced with preparing a written report of his findings. Each discussion is replete with practical advice and many pitfalls are pointed out for the benefit of the unwary. The illustrations are ample and their effect is extended by the use of numerous small sketches.

The book suffers from the lack of a well arranged bibliography. Many references are scattered throughout the text and are thus not easily accessible. Some special procedures such as encephalography, cerebral angiography and myelography are not included. These latter omissions in general do not detract from the value

of the work since the purpose of the author was to establish the fundamentals of film interpretation rather than to describe details of special procedures.

The volume is written on good quality paper, is well bound and has an attractive appearance.

This book should be invaluable to hospital residents and students who are just beginning their film reading careers. To the more experienced roentgenologist it offers easy but profitable reading.

R. N. C.

*Operative Obstetrics*, 5th edition. By J. M. Munro Kerr and J. Chassar Moir. 948 pp. $12.00. *The Williams & Wilkins Co., Baltimore, Maryland*

As stated in the preface this is a volume for the obstetric specialist. It has been largely rewritten and the order of presentation revised. The presentation of material is divided into six sections each containing a variable number of chapters. The first concerns the management of normal labor and is rightly short for the volume is chiefly concerned with operative problems. This part does contain a chapter on anesthetics, analgesics and sedatives. While this chapter goes into considerable detail as to methods and drugs, there is little or no emphasis on the importance of proper selection for various obstetric difficulties and procedures, although this is occasionally discussed later in the text.

The next three sections take up dystocia from the point of view of forces, fetus and parturient canal respectively. This entire section is excellently done. It is simple, concise and thoroughly modern in its approach. The references, which incidentally are readily accessible at the bottom of each page, are carefully selected and represent the outstanding contributions to the subject at hand.

The next section on operative procedures is extremely complete both in text and illustration. The inclusion of pubiotomy and accouchement forcé might be open to question, as they are no longer acceptable procedures in this country at least. However, in most instances the points of view and practice between England and the United States are strikingly similar.

The last section on accidents to mother and child is informative. Particularly so is the last chapter on factors influencing infant and maternal mortality.

This volume is without doubt a real contribution to the obstetric art.

Louis M. Hellman

*Liver Injury*. Transactions of the Eighth Conference April 28–29, 1949. Edited by F. W. Hoffbauer. 164 pp. $1.60. *Josiah Macy Jr. Foundation, New York.*

Like its predecessors, the current volume of transactions of the Conference on Liver Injury is a record of the round table discussions of this stimulating group of investigators. The topics discussed included the circulation of the hepatic lobule, the effect of ligation of the hepatic artery, the dietetic production of massive hepatic necrosis, the effect of the liver on the hormonal control of the circulation, the metabolic behaviour of the eviscerate rat, and the pathogenesis of cirrhosis

of the liver. A discussion of the effects of the administration of carbohydrate in clinical and experimental hepatic damage demonstrated the conflicting evidence on which our therapeutic dogmas rest. One is impressed with the fact that isotopes are apparently little used by the investigators who participated in the conference. This little volume makes lively reading. Its utility might be improved by the inclusion of an index.

O. D. R.

*Lehrbuch der Verdauungskrankheiten.* By NORBERT HENNING and WALTER BAUMANN. 799 pp. 68 marks. *Georg Thieme Verlag, Stuttgart.*

This German text-book is a comprehensive treatment of gastroenterology, attractively printed and illustrated. There are numerous X-rays, the work of Dr. Henning's collaborator, Walther Baumann. This text-book is useful as an authoritative summary of current views on gastro-enterology in Europe. The reader is surprised to find that these views are not particularly different from those widely held in this country. It is easy to translate the text into English, so that this book should be useful to anyone wishing to familiarize himself with recent European literature on gastroenterology. A surprising number of American references are cited too. If more American literature could be included, this book might profitably be translated and published in an English language edition.

O. D. R.

*Practical Neurological Diagnosis,* 4th edition. By R. GLEN SPURLING. 268 pp. $5.00. *Charles C Thomas, Springfield, Illinois.*

This very useful little text on neurological techniques for students has been considerably revised in the fourth edition without, as so often happens, increasing the bulk of the book. The author has rewritten the chapter on the cerebellum bringing it up to date. A small section on the hypothalamus has been added as well as a glossary of neurological terms.

The text explains the techniques of clinical neurological examination and discusses clearly the abnormalities found in various disease states. The ancillary procedures—lumbar puncture, roentgenography, pneumoencephalography and ventriculography—are well presented but one misses a section on cerebral angiography, a procedure which is daily becoming more useful.

The glossary of neurological terms should be useful but the definitions given for a number of terms do not seem to be quite appropriate. One might mention convulsive discharge, convulsant equivalent, decerebrate rigidity and decomposition of movement as examples.

The book has been nicely set up—a tribute to the publisher's tastes—and is pleasing to the eye. The textbook can be highly recommended to all medical students and graduates desirous of gaining further information of clinical neurology.

A. E. W.

*Conference on Problems of Aging.* Transactions of the Tenth and Eleventh Conferences, Feb. 9–10, 1948, and April 25–26, 1949. Edited by NATHAN W. SHOCK. 258 pp. $3.75. *Josiah Macy, Jr. Foundation, New York.*

The sense of accomplishment that can be extracted from these minutes of the Tenth and Eleventh Conferences on Problems of Aging resides chiefly in appreciation of the broad canvas splashed by the conferees. Formal papers were few and discussion was vigorous and uninhibited, but the panorama considers social, economic, emotional and metabolic facets.

While much of the material has general interest, even beyond the scope of gerontology—particularly the practical questions concerning care and handling of experimental animals—, the uninitiated must plow thoroughly to unearth specific information. One may, therefore, wonder at the wisdom and scientific utility of such completely stenographic reports as this. The conferences were valuable; the conferees had a good time. But the literal record is uneconomical, sometimes stimulating, sometimes discouraging, and permanent evidence that the art of conversation and the art of writing are not one.

K. L. Z.

*Water and Salt Depletion.* By H. L. MARRIOTT. 80 pp. $2.00. *Charles C Thomas, Springfield, Illinois.*

This addition to the monograph series, American Lectures in Physiology, is the work of a distinguished British clinician. In a field in which focus is often shifted and in which truth is still on the loose, Dr. Marriott has elected the clinical and practical view. There may be occasional challenges of physiological interpretation, but the aim has been achieved, and the monograph demonstrates decisively the fundamental contributions distillable from careful, accurate and simple observation of patients. The salt depletion of which Dr. Marriott writes is implicitly sodium chloride, and his recommended repletive is sodium chloride; his basic thesis is maintenance of isosmolarity. There are reasons to doubt, however, that this is the full view, and one recalls, on the one hand, patients who died with "normal blood chemistries," and, on the other hand, the sustaining effects of potassium in certain states of water and salt depletion.

K. L. Z.

*Urological Surgery,* 2nd edition. By AUSTIN INGRAM DODSON (with contributions by 12 other authors) 855 pp. $13.50. *The C. V. Mosby Co., St. Louis, Missouri.*

This single volume text on surgical principles and techniques in urology is a second edition of an already accepted work. It has been revised, enlarged, and rewritten in the light of recent advances. It is a useful but not perfect book. The material is sometimes spottily covered and there is occasional overlapping due to incomplete editing of the material offered by some of the contributors. In some sections the book seems padded with material which is not particularly essential to a text on operative urology.

There are 48 chapters covering most of the field of urological surgery from circumcision to cystectomy including recent advances, with a few exceptions. The importance of intravascular hemolysis in transurethral surgery is barely mentioned. The excellent section on plastic operations for hypospadias repair omits the currently popular "Dennis Brown procedure." The otherwise complete chapter on ureteral transplantations omits general consideration of the advantages and disadvantages of the various types of ureteroenterostomy, and the currently popular "end-to-side" anastomoses of ureter and bowel are not discussed. Trans-thoracic renal surgery is not discussed.

The book is at its best when the author describes his own experiences and refers to his own considerable published works. It is profusely illustrated. Some of the x-rays are poorly reproduced and repetitious. The many original and copied drawings of familiar surgical techniques by Helen Lorraine are for the most part well done. They are often as helpful in the understanding of a procedure as the text; and in some instances the drawings explain a whole operation more clearly than words (e.g., ureteroureterostomy, page 429).

This text should be useful to all urological surgeons eager to improve their techniques and to learn new ones. It should be most helpful to young men in training. Although this is probably the best available single book on operative urology at present, the definitive text of this sort remains yet to be written.

W. E. G.

## BOOKS RECEIVED FOR REVIEW

*Annual Review of Medicine*, vol. 1. Edited by W. C. CUTTING and H. W. NEWMAN assisted by A. BLALOCK, J. S. L. BROWNE, A. M. BUTLER, E. M. MACKAY and S. C. MADDEN. 484 pp. $6.00. *Annual Reviews, Inc., Stanford, California.*

*Anxiety in Pregnancy and Childbirth*. By HENRIETTE R. KLEIN, HOWARD W. POTTER and RUTH B. DYK. 111 pp. $2.75. *Paul B. Hoeber, Inc., New York.*

*Brain and Behavior*. By N. E. ISCHLONDSKY. 182 pp. $7.00. *The C. V. Mosby Co., St. Louis, Missouri.*

*Delayed Union in Fractures of the Long Bone*. By KENNETH W. STARR. 215 pp. $9.00. *The C. V. Mosby Co., St. Louis, Missouri.*

*The Illustrations from the Works of Andreas Vesalius*. Edited by J. B. DEC. M. SAUNDERS and CHARLES D. O'MALLEY. 248 pp. $10.00. *The World Publishing Co., Cleveland, Ohio.*

*Lehrbuch Der Rontgenologischen Differentialdiagnostik*. By W. TESCHENDORF. 779 pp. DM. 84. *George Thieme Verlag, Stuttgart.*

*Lehrbuch Der Rontgenologischen Differentialdiagnostik*. By W. TESCHENDORF. 608 pp. DM. 72. *George Thieme Verlag, Stuttgart.*

*Lipidoses. Diseases of the Cellular Lipid Metabolism*. By SIEGFRIED J. THANNHAUSER. Edited by HENRY A. CHRISTIAN. Reprinted from Oxford Loose-Leaf Medicine. 605 pp. $12.00. *Oxford University Press, New York.*

*A Primer for Diabetic Patients*, 9th edition. By RUSSELL M. WILDER. 200 pp. $2.25. *W. B. Saunders Co., Philadelphia, Pa.*

*Woman's Surgeon. The Life Story of J. Marion Sims*. By SEALE HARRIS. 432 pp. $5.00. *The Macmillan Company, New York.*